PATENT COOPERATION TREATY

REC'D 06 NOV 2000
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

15

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
92513/PRS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/02271	14/07/1999	14/07/1998
International Patent Classification (IPC) or national classification and IPC C01G1/00		
Applicant CAMBRIDGE DISPLAY TECHNOLOGY LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☐ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/02/2000	02.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mayne, J Telephone No. +49 89 2399 8572

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/02271

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

D scription, pages:

1-28 as originally filed

Claims, No.:

1-37 as originally filed

Drawings, sheets:

1/21-21/21 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-27, 36.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-27, 36.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The Applicant filed a supplementary set of drawings, sheets 1/27-27/27, with WIPO in October 1999. Of these only Figs 1-20 (sheets 1/27-22/27) correspond to drawings which were originally filed. Sheets 23/27-27/27 cannot be unambiguously derived from the application as originally filed. Article 34(2)(b) PCT is therefore infringed.
On the demand form PCT 401, received 7.2.00, the Applicant has indicated that the drawings as originally filed are to be used for the purposes of examination. Sheets 23/27-27/27 are there not regarded for the present opinion.

It is not clear what is meant by claim 1.
If a mixture is washed with a solvent in which the nanoparticles are soluble it is not apparent how they remain as "nanoparticles in the solvent". If solid matter is dissolved it does not remain as solid matter.
Hence no opinion on novelty and inventive step can be given on claim 1, its dependent claims and claims which refer back to claim 1, i.e. claims 1-27.

It is not known what is the scope of claim 36 so that also no opinion on novelty and inventive step can be given on claim 36.

Re Item IV

Lack of unity of invention

Inasfar as the claims can be understood, the independent claims can be grouped into the following catagories:

Group A

Claim 1 concerns a method for preparing nanoparticles by washing a mixture of nanoparticles and another material with a solvent in which the nanoparticles are soluble to remove the said other material and form a solution of the nanoparticles in the solvent.

Claim 24 concerns a solution of nanoparticles formed by a method according to claims 1-20.

Claim 25 concerns a polymer precursor material containing nanoparticles formed by a method according to claim 21.

Claim 26 concerns a polymer material containing a substantially uniform dispersion of nanoparticles formed by a method according to claims 1-20.

Claim 27 concerns a polymer material containing a substantially uniform dispersion of nanoparticles formed by a method according to claims 22-23.

Claim 36 concerns a method for forming a solution of nanoparticles "substantially as herein described with reference to the accompanying drawings".

Group B

Claim 28 concerns an organic material containing a substantially uniform dispersion of nanoparticles.

Claim 35 concerns a method for tailoring at least one property of an organic material, the method comprising forming a substantially uniform dispersion of nanoparticles in the organic material.

Claim 37 concerns an organic material containing a substantially uniform dispersion of nanoparticles "substantially as herein described".

The present application does not fulfill the requirements of Rule 13.1 PCT. The reasons are as follows:

The only common concept linking groups A and B is nanoparticles. This concept is already known, as acknowledged in the discussion of the background art in the application, see also, for example, DE-A-4133621, DE-A-19540623.

As discussed on PCT form 405 of 17.4.00 this preliminary opinion will be drawn up only on claims of Group A, since no reply has been received within the time limit set (1 month).

Re Item VII

Certain defects in the international application

The PCT does not recognize "incorporated by reference", p. 3, last paragraph.
The Application refers to unpublished citations, p. 4, 3rd paragraph.

Re Item VIII

Certain observations on the international application

Article 6 PCT

Claims 12 and 13 contradict claim 1 on which they depend. If the said other material is soluble in the solvent (claim 12) as well as the nanoparticles (claim 1) it is not clear how the intended "preparation of nanoparticles for use", i.e. separation, (claim 1) could occur.

The indication in the description (p. 5, 1st paragraph) of the use of a solvent that dissolves the other material but not the nanoparticles also contradicts claim 1.

There is discrepancy between claims 1 and 26. Claim 26 refers to a dispersion of nanoparticles formed by a method according to claim 1. However, claim 1 makes no mention of a dispersion of nanoparticles.

It is stated on p. 8 (2nd paragraph) that the density of the nanoparticles distribution in the body is "preferably greater than 10^{-17} and/or less than 10^{-19} /cm^2. A number which is greater than 10^{-17} cannot be less than 10^{-19}.

TENT COOPERATION TRE. Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 June 2000 (15.06.00)	
International application No. PCT/GB99/02271	**Applicant's or agent's file reference** +2513/PRS
International filing date (day/month/year) 14 July 1999 (14.07.99)	**Priority date** (day/month/year) 14 July 1998 (14.07.98)
Applicant HO, Peter et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 February 2000 (07.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Pascal Piriou

Telephone No.: (41-22) 338.83.38

TENT COOPERATION TRE Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

SLINGSBY, Philip, Roy
Page White & Farrer
54 Doughty Street
London WC1N 2LS
ROYAUME-UNI

Date of mailing (day/month/year) 15 June 2000 (15.06.00)	
Applicant's or agent's file reference +2513/PRS	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/02271	**International filing date** (day/month/year) 14 July 1999 (14.07.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
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TENT COOPERATION TRE Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

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ROYAUME-UNI

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15 June 2000 (15.06.00)

Applicant's or agent's file reference
+2513/PRS

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/02271

International filing date (day/month/year)
14 July 1999 (14.07.99)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

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Israel

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State of Residence: IL
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Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

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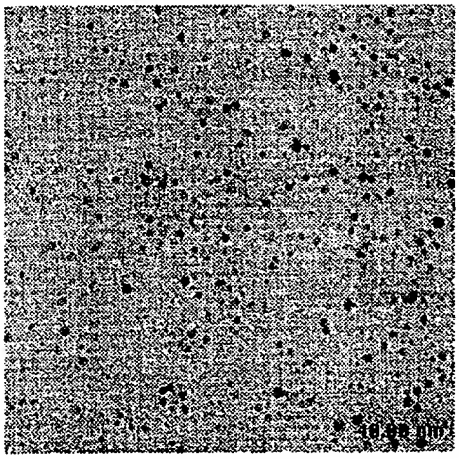
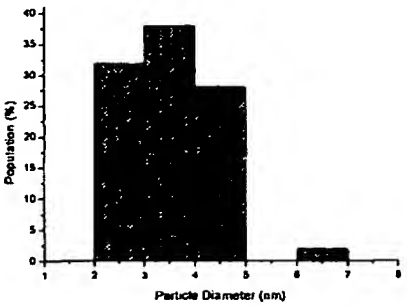
[Continued on next page]

(54) Title: COLLOID SOLUTION OF METAL NANOPARTICLES, METAL-POLYMER NANOCOMPOSITES AND METHODS FOR PREPARATION THEREOF





(57) Abstract: A metal nanoparticle colloid solution, metal-polymer nanocomposites, and methods for preparing the same are provided. The metal nanoparticle colloid solution and the metal-polymer nanocomposites can be prepared with a variety of polymeric stabilizers and have uniform particle diameter and shape. The metal nanoparticle colloid solution and the metal-polymer nanocomposites have wide applications, for example, as an antibacterial agent, a sterilizer, a conductive adhesiv, conductive ink, or an electromagnetic wave shielder for an image display.

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Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

COLLOID SOLUTION OF METAL NANOPARTICLES, METAL-POLYMER NANOCOMPOSITES AND METHODS FOR PREPARATION THEREOF

Technical Field

The present invention relates to a colloid solution of metal nanoparticles, metal-polymer nanocomposites, and methods for preparing the same, and more particularly, to a metal colloid solution and metal-polymer nanocomposites prepared using a variety of polymeric stabilizers and having a uniform particle diameter, and methods for preparing the same.

Background Art

Recently, a method for preparing a colloidal dispersion of silver nanoparticles using gamma rays and appropriate stabilizers, such as polyvinyl alcohol and sodium dodecyl sulfate (SDS) was disclosed (*Nature* 1985, *317*, 344; *Materials Letters* 1993, *17*, 314). The preparation method using gamma rays was reported to provide uniform diameter distribution of the silver nanoparticles. The metal nanoparticles prepared by those methods were known to have a size of from about 8 nm to tens of nanometers from the outstanding research reports. However, the metal nanoparticles are prepared by these methods not so desirable in terms of particle diameter and shape uniformity.

It is important to obtain pure silver particles having a uniform shape within a narrow distribution range of particle diameters for industrial applications. For example, ultrafine silver particles are essential materials in the electronics applications, for example, for conductive ink and paste and adhesive applied in the manufacture of a variety of electronic parts.

As described above, there is a need for a new method for preparing metal nanoparticles having a uniform size and shape. In addition, good dispersion stability for preventing agglomeration of metal nanoparticles in a dispersion medium is another consideration for industrial applications. For diversified industrial applications, miscibility with a variety of organic solvents, plasticizers,

and resins is required to prepare a metal colloid solution in a non-aqueous medium.

A variety of methods for preparing a solid phase of polymer-metal nanocomposites were suggested (*Polym. Composites* 1996, *7*, 125; *J. Appl. Polym. Sci.* 1995, *55*, 371; *J. Appl. Polym. Sci.* 1996, *60*, 323). These methods involve two steps: (1) polymerization of monomer particles and (2) reduction of metal ions in a polymerized medium. However, the separate polymerization and reduction processes cause non-uniform size distribution of the metal nanoparicles in the polymerized medium.

To solve this problem, a method for preparing silver-polymer nanocomposites using gamma rays was developed (*Chem. Commun.* 1997, 1081). In the method, a silver salt is dissolved in water, mixed with acrylic amide as a water-soluble monomer, and subjected to gamma-rays irradiation to prepare the silver-polymer nanocomposites. Here, reduction of silver ions coincides with polymerization of the monomer, so that the metal nanoparticles are comparatively uniformly dispersed in the polymerized medium.

However, this method also cannot be applied when using a variety of water-insoluble monomers. To overcome the limitation encountered when using an aqueous medium, the preparation of silver-polymer nanocomposites from a water-in-oil (W/O) emulsion was reported (*Chem. Commun.* 1998, 941), wherein toluene was used for the oil phase.

According to the method, since a variety of water-insoluble monomers can be applied, various kinds of metal-polymer nanoparticles can be prepared. However, the use of excess toluene for the oily medium, up to about 5 times the amount of water, causes environmental concerns. In addition, a safe working environment is not guaranteed due to a high risk of explosion in its preparation.

Disclosure of the Invention

Accordingly, it is an object of the present invention to provide a colloid solution of metal nanoparticles having a uniform particle characters and a method for preparing the same.

It is another object of the present invention to provide metal-polymer nanocomposites having a uniform particle characters and a method for preparing the same.

In one aspect, the present invention provides a method for preparing a metal nanoparticle colloid solution, comprising: dissolving a metal salt and a water-soluble polymer in water, a non-aqueous solvent, or a solvent mixture of water and a non-aqueous solvent; purging a reaction container containing the solution with nitrogen or argon gas; and radiating radioactive rays onto the solution.

In the preparation method, the water-soluble polymer includes polyvinyl pyrrolidone, a copolymer having vinyl pyrrolidone as a first polymerization unit, and a fatty acid-substituted or unsubstituted polyoxyethylene. The copolymer having vinyl pyrrolidone as the first polymerization unit includes (1-vinyl pyrrolidone)-acrylic acid copolymer, (1-vinyl pyrrolidone)-vinyl acetic acid copolymer, (1-vinyl pyrrolidone)-styrene copolymer, and (1-vinyl pyrrolidone)-vinyl alcohol copolymer. The fatty acid-substituted polyoxyethylene includes polyoxyethylene stearate and polyoxyethylene palmitate.

In another aspect, the present invention provides a metal nanoparticl colloid solution prepared by the preparation method described above.

In another aspect, the present invention provides a method for preparing metal-polymer nanocomposites, comprising: dissolving a metal salt and a polymeric stabilizer in a solvent mixture of water and a non-aqueous solvent; purging a reaction container containing the solution with nitrogen or argon gas; and radiating radioactive rays onto the solution to obtain precipitates.

In the preparation method of the metal-polymer nanocomposites, the polymeric stabilizer is at least one polymer selected from the group consisting of polyethylene, polyacrylonitrile, poly(methyl (meth)acrylate), polyurethane, polyacrylamide, and polyethylene glycol.

According to the present invention, the colloid solution of metal nanoparticles and the metal-polymer nanocomposites have favorable stability, a uniform shape, and a small diameter within a narrow distribution range, so that

the colloid solution of metal nanoparticles and the metal-polymer nanocomposites have wide, effective applications, for example, as an antibacterial agent, a deodorizing agent, a conductive adhesive, conductive ink, and a electromagnetic wave shielder for an image display.

The formation of the silver nanoparticles will be described in greater detail. Electrons are generated in a solvent by gamma-rays irradiation and reduce silver ions in a solution. Reduced silver atoms agglomerate to form a silver cluster and become larger. In this case, when an appropriate polymeric stabilizer is added, the agglomeration of the silver atoms can be prevented to result in nano-sized silver particles. Polymeric stabilizers stabilize the nanoparticles in a colloid state through steric repulsion as well as prevent the silver clustering. The gamma-rays irradiation produces radicals as well as the electrons in the solvent. To remove the radicals, a scavenger, such as alcohol, is used. Oxygen present in the solution is removed by nitrogen or argon purging before the gamma-rays irradiation, to prevent side reactions by the oxygen.

To prepare the colloid solution of metal nanoparticles according to the present invention, any metal salt capable of forming a general nanoparticle colloid solution can be used without limitations. However, in terms of conductivity and economical reasons, a salt of at least one metal selected from the group consisting of silver, copper, nickel, palladium, and platinum is preferable, with the silver salt being more preferable.

The metal salt is, for example, nitrate, sulfate, hydrochloride, perchlorate, or acetate. According to the present invention, a silver salt, such as $AgNO_3$, $AgClO_4$, Ag_2SO_4, or CH_3COOAg is more preferred. These silver salts are well dissolved in water and thus form an aqueous colloid of silver nanoparticles.

In the preparation of the colloid solution of metal nanoparticles according to the present invention, a water-soluble polymer, preferably, having a weight average molecular weight of 2,000-2,000,000, is used as a stabilizer for improving dispersion of the metal nanoparticles. Suitable stabilizers include, for example, polyvinyl pyrrolidone, a copolymer including vinyl pyrrolidone as a first polymerization unit, and a fatty acid-substituted or unsubstituted polyoxyethylene.

The copolymer including vinyl pyrrolidone as a first polymerization unit may further include an acrylic acid, styrene, vinyl acetate, or vinyl alcohol as a second polymerization unit. Examples of the copolymer include (1-vinyl pyrrolidone)-acrylic acid copolymer and (1-vinyl pyrrolidone)-vinyl acetic acid copolymer. The copolymer includes the first and second polymerization units in a weight ratio of 1:99-99:1, and preferably, 20:80-80:20. Preferably, the (1-vinyl pyrrolidone)-acrylic acid copolymer includes a 1-vinyl pyrrolidone repeating unit and an acrylic acid repeating unit in a weight ratio of 75:25. Preferably, the (1-vinyl pyrrolidone)-vinyl acetic acid copolymer includes a 1-vinyl pyrrolidone repeating unit and a vinyl acetic acid repeating unit in a weight ratio of 57:43.

Regarding the fatty acid-substituted polyoxyethylene, which is a wat r-soluble polymer used as the stabilizer, the fatty acid is palmitic acid, oleic acid, linoleic acid, or stearic acid, with the stearic acid being more preferred.

Any solvent capable of dissolving the water-soluble polymer and metal salt therein can be used without limitations. For example, water, a non-aqueous solvent, or a mixture of these solvents can be used. Suitable non-aqueous solvents include alcoholic solvents, and typically, isopropyl alcohol, methanol, ethanol, ethylene glycol, or a mixture including at least two of the forgoing solvents.

The non-aqueous solvents also act as a scavenger for removing radicals during gamma-rays radiation as well as act as a solvent for the metal salt and water-soluble polymer.

According to the present invention, the water-soluble polymer is used in an amount of 0.1-10 parts by weight based on 100 parts of the solvent by weight. If the water-soluble polymer is used in an amount of less than 0.1 parts by weight, it is difficult to provide the effect of the stabilizer. If the water-soluble polymer is used in an amount of greater than 10 parts by weight, the particle size undesirably increases.

According to the present invention, the metal salt is used in an amount of 0.01-5 parts by weight based on 100 parts of the solvent by weight. If the metal salt is used in an amount of less than 0.01 parts by weight, it is difficult to provide

the effect of the metal salt. If the metal salt is used in an amount of greater than 5 parts by weight, the particle size increases, or the particles slightly precipitate.

In the preparation of the colloid solution of metal nanoparticles according to the present invention, a water-soluble polymer and a metal salt are dissolved in a solvent. A reaction container containing the solution is purged with nitrogen (N_2) or argon (Ar) gas for 10 minutes to 10 hours and tightly sealed.

Next, the resultant product is irradiated with radioactive rays, and preferably, gamma rays, to a radiation dosage of 10-50 KGy. As a result, the colloid solution of metal nanoparticles having a much smaller particle diameter of about 1-5 nm than those prepared by conventional methods, within a narrow distribution of particle diameters, is obtained.

In the colloid solution of metal nanoparticles prepared by the method according to the present invention, a post-process of diluting the source solution and ultrasonic treatment may be performed to decompose the metal nanoparticles further into much smaller metal particles. The post-process supports the fact that the adsorption and steric repulsion mechanism of polymers enables the formation of the metal nanoparticles and ensures dispersion stability. In particular, very small metal nanoparticles are surrounded and adsorbed by the polymeric stabilizer to form clusters of the polymeric stabilizer-adsorbed metal nanoparticles. Since the clusters of the metal nanoparticles agglomerate, the metal nanoparticles forming the colloid appear to be much larger after the radioactive-rays irradiation. Accordingly, when the colloid of the m tal nanoparticles is diluted and subjected to the ultrasonic treatment, the clusters of the metal nanoparticles are decomposed further into much smaller metal particles.

In the present invention, the much smaller particle diameter and narrower distribution of particle diameters, compared to when conventional methods are applied, are believed to be due to the use of the water-soluble polymeric stabilizer, such as polyvinyl pyrrolidone, (1-vinyl pyrrolidone)-acrylic acid copolymer, polyoxyethylene stearate, and (1-vinyl pyrrolidone)-vinyl acetic acid copolymer.

The metal nanoparticles having a very small diameter prepared in the present invention have a very large surface area-to-volume ratio, and thus they

provide good antibacterial activity and conductivity even when only a trace is used. Therefore, the colloid solution of the metal nanoparticles according to the present invention can be used as an antibacterial agent; a sterilizer, a deodorizing agent, an electromagnetic wave shielder, and conductive adhesive and ink.

For diversified industrial applications, the metal nanoparticles according to the present invention need to be miscible with a variety of organic solvents, plasticizers, and resins to prepare a non-aqueous colloid solution of the metal nanoparticles. In this case, a non-aqueous solvent, which does not contain water, i.e., an alcoholic solvent, can be used alone as the solvent. The alcoholic solvent acts as a scavenger as well as the solvent, and thus is favorable for economical reasons. Among the above-listed kinds of alcoholic solvents, the ethylene glycol is more preferred as the solvent and scavenger.

For the miscibility with a variety of resins, plasticizers, and solvents, instead of the ethylene glycol used as a non-aqueous alcohol, isopropyl alcohol can be used as the solvent and scavenger. In this case, the metal nanoparticles are miscible with alcohol-soluble resins, alcohol-soluble plasticizers, such as dioctyl phthalate (DOP), and organic solvents.

In another aspect, the present invention provides a solid paste of metal-polymer nanocomposites. The solid paste of the metal-polymer nanocomposites is prepared by a similar method as that applied to prepare the colloid solution of the metal nanoparticles as described above, except that polyacrylamide or polyethylene glycol is used as a polymeric stabilizer. The polyacrylamide and polyethylene glycol are water-soluble polymers and precipitate the metal-polymer nanocomposites when dissolved in a solvent together with a metal salt, followed by radioactive-rays irradiation.

In the preparation of the solid paste of the metal-polymer nanocomposites, when a water-insoluble stabilizer, such as poly(methyl (meth)acrylate), polyacrylonitrile, or polyurethane, is used, a surfactant, for example, polyoxyethylene sorbitan mono-oleate, which is commercially available in the trade name of Span-80, Tween-81, or Tween-80, is added. In this case, it is preferable to initially form an emulsion with the addition of the surfactant. The

surfactant is added little by little until the emulsion is formed.

As in the preparation of the colloid solution of the metal nanoparticles, in the preparation of the solid paste of the metal-polymer nanocomposites, it is preferable to use a mixture of water and a non-aqueous solvent as the solvent, instead of using water or the non-aqueous solvent alone.

In the preparation of the solid paste of the metal-polymer nanocomposites, it is preferable that the metal salt is added in an amount of 0.01-5 parts by weight based on 100 parts of the solvent by weight. If the metal salt is added in an amount of less than 0.01 parts by weight, the effect of adding the metal salt is negligible. If the metal salt is added in an amount of greater than 5 parts by weight, the particle size increases.

In the preparation of the metal-polymer nanocomposites according to the present invention, the polymeric stabilizer is added in an amount of about 0.1-10 parts by weight based on 100 parts of the solvent by weight. If the amount of the polymeric stabilizer is less than 0.1 parts by weight, the effect of adding the polymeric stabilizer is negligible. If the amount of the polymeric stabiliz r exceeds 10 parts by weight, the particle size increases, and the addition of the polymeric stabilizer such an amount is uneconomical.

In the preparation of the metal-polymer nanocomposites according to the present invention, the polymeric stabilizer and metal salt are dissolved in a solvent, and a reaction container containing the solution is purged with nitrogen or argon gas for 30 minutes to 10 hours and completely tightened. Next, the solution is irradiated with gamma rays of a radiation dosage of about 10-50 KGy, followed by solvent removal and vacuum drying to attain the metal-polymer nanocomposites according to the present invention.

The metal-polymer nanocomposites according to the present invention have a uniform particle diameter at room temperature. Since greatly diversified kinds of polymers can be applied to the metal-polymer nanocomposites, unlike conventional methods using monomers to prepare metal-polymer nancomposites, it is easy to control the molecular weight. In addition, due to a great surface area-to-volume ratio of the metal-polymer nanocomposites, favorable effects, for

example, in terms of antibacterial activity and conductivity, are provided with a trace of the metal-polymer nanocomposites. The metal-polymer nanocomposites can be effectively used as an antibacterial agent, a sterilizer, a deodorizing agent, a conductive adhesive, and conductive ink.

Brief Description of the Drawings

FIG. 1 shows a transmission electron microscopic (TEM) photograph and particle diameter distribution of silver nanoparticles prepared in Example 1 according to the present invention;

FIG. 2 shows the UV/VIS absorption spectrum of the silver nanoparticles prepared in Example 1 according to the present invention at 405 nm;

FIG. 3 is a TEM photograph after dilution with water and ultrasonic treatment of the silver nanoparticles prepared in Example 2 according to the present invention;

FIG. 4 shows a TEM photograph and particle diameter distribution of silver nanoparticles prepared in Example 5 according to the present invention;

FIG. 5 shows the UV/VIS absorption spectrum of the silver nanoparticles prepared in Example 5 according to the present invention at 405 nm;

FIG. 6 is a field emission scanning electron microscopic (FESEM) photograph of a paste of silver-polymer nanocomposites prepared in Example 6 according to the present invention;

FIG. 7 shows a TEM photograph and particle diameter distribution of a dispersion of silver-polymer nanocomposites prepared in Example 7 according to the present invention in chloroform;

FIG. 8 shows the UV/VIS absorption spectrum of the silver-polymer nanocomposites prepared in Example 7 according to the present invention at 405 nm;

FIG. 9 is a TEM photograph of a silver nanoparticle colloid solution prepared in Example 1 according to the present invention after being left for 10 months at room temperature;

FIG. 10 shows the infrared (IR) spectrum of a silver nanoparticle colloid

solution prepared in Example 2 according to the present invention;

FIG. 11 shows the surface enhanced Raman scattering spectrum of the silver nanoparticles prepared in Example 2 according to the present invention with respect to pH of a 1.0×10^{-5} M thionin solution; and

FIG. 12 shows the result of an antibacterial activity test of a textile soaked with the silver nanoparticle colloid solution prepared in Example 2 according to the present invention; and

FIG. 13 shows the result of an antibacterial activity test of a textile soaked with a solution containing no silver nanoparticles according to the present invention.

Best mode for carrying out the Invention

The present invention will be described in greater detail with reference to the following examples. The following examples are for illustrative purposes and are not intended to limit the scope of the invention.

Example 1: Silver nanoparticle colloid solution prepared by using (1-vinyl pyrrolidone)-acrylic acid copolymer as a stabilizer

1.863 g $AgNO_3$, 395 g isopropyl alcohol, and 11.137g (1-vinyl pyrrolidone)-acrylic acid copolymer in a weight ratio of 75:25, having a molecular weight (MW) of 96,000, were thoroughly dissolved in 592 g water. A reaction container containing the solution was purged with nitrogen gas for 1 hour and completely tightened, followed by gamma-rays radiation of a dosage of 30 KGy, thereby to prepare a yellow silver nanoparticle colloid solution.

Particle diameter distribution and particle shape were observed for the prepared silver nanoparticle colloid solution by using a transmission electron microscope (TEM). The results are shown in FIG. 1.

As shown in FIG. 1, the silver nanoparticle colloid solution had a very uniform particle diameter distribution and a uniform particle shape. Most of the particles had a diameter of 3.0 ± 0.9 nm on average, which is the smallest among silver nanoparticles prepared by gamma-rays irradiation, which have been

reported to date.

The formation of the silver nanoparticles was identified by UV/VIS spectrometry. The result is shown in FIG. 2. As shown in FIG. 2, an absorption peak of the silver nanoparticles appeared at 405 nm.

Example 2: Silver nanoparticle colloid solution prepared by using polyvinyl pyrrolidone as a stabilizer

A silver nanoparticle colloid solution was prepared in the same manner as in Example 1, except that 11.137 g polyvinyl pyrrolidone having a MW of 55,000 was used as the stabilizer, instead of the (1-vinyl pyrrolidone)-acrylic acid copolymer. The resultant silver nanoparticle colloid solution had a minimum particle diameter of 6.6±1.1 nm and an average particle diameter of about 10-12 nm.

Example 3: Silver nanoparticle colloid solution prepared by using polyoxyethylene stearate as a stabilizer

A silver nanoparticle colloid solution was prepared in the same manner as in Example 1, except that 11.137 g polyoxyethylene stearate having a MW of ~ 2,000 was used as the stabilizer, instead of the (1-vinyl pyrrolidone)-acrylic acid copolymer. The resultant silver nanoparticle colloid solution had an average particle diameter of 7.5±1.8 nm.

Example 4: Particle diameter of silver nanoparticle colloid solution prepared by using polyvinyl pyrrolidone as a stabilizer after dilution and ultrasonic treatment

The silver nanoparticle colloid solution (having an average particle diameter of 12.1±1.6 nm) prepared in Example 2 was diluted 20 folds with water and subjected to ultrasonic treatment for 3 hours and particle diameter measurement. The result is shown in FIG. 3. As shown in FIG. 3, after the dilution and the ultrasonic treatment, particles of a diameter of ~2 nm and ~ 4 nm appeared. This result supports that the particle diameter can be further reduced

by dilution and ultrasonic treatment. Apparently, a number of very small unit silver nanoparticles on which polyvinyl pyrrolidone is adsorbed form the silver nanoparticle colloid solution.

Example 5: Silver nanoparticle colloid solution prepared by using ethylene glycol as a solvent and polyvinyl pyrrolidone as a stabilizer

A non-aqueous, yellow silver nanoparticle colloid solution was prepared in the same manner as in Example 1, except that 987g ethylene glycol was used, instead of the isopropyl alcohol and water.

Particle diameter and particle diameter distribution were observed for the prepared silver nanoparticle colloid solution by using a transmission electron microscope (TEM). The results are shown in FIG. 4. As shown in FIG. 4, the silver nanoparticle colloid solution had a very uniform particle diameter distribution and a small, uniform particle diameter of 6.02±0.8 nm on average.

The formation of the silver nanoparticles was identified by UV/VIS spectrometry. The result is shown in FIG. 5. As shown in FIG. 5, an absorption peak of the silver nanoparticles appeared at 405 nm.

Example 6: Solid paste of silver-polyacrylamide nanocomposit s prepared by using polyacrylamide as a stabilizer

592 g water, 1.863 g $AgNO_3$, and 395 g isopropyl alcohol were mixed together, and 11.137 g polyacrylamide was added to the mixture and vigorously stirred. A reaction container containing the solution was purged with nitrogen gas for 1 hour and completely tightened, followed by gamma-rays radiation of a dosage of 30 KGy, thereby to attain a paste of precipitates. The solvent was removed from the paste, followed by vacuum drying. As a result, silver-polyacrylamide nanocomposites were obtained. The dried silver-polyacrylamide nanocomposites were dispersed in water.

The solid paste of the silver-polyacrylamide nanocomposites was observed by field emission scanning electron microscopy (FESEM). The result is shown in FIG. 6. As shown in FIG. 6, the silver-polyacrylamide nanocomposites had a

particle diameter of 4-8 nm and a uniform particle shape.

Example 7: Solid paste of silver-poly(methyl methacrylate) nanocomposites prepared by using poly(methyl methacrylate) as a stabilizer

592 g water, 1.863 g $AgNO_3$, and 395 g isopropyl alcohol were mixed together, and 11.137 g poly(methyl methacrylate) was added to the mixture and vigorously stirred. Twin-81 as a surfactant was added little by little to the mixture with stirring until a white emulsion is formed. A reaction container containing the emulsion was purged with nitrogen gas for 1 hour and completely tightened, followed by gamma-rays radiation of a dosage of 30 KGy, thereby to attain a solid paste of precipitates. The solvent was removed from the paste, followed by vacuum drying. As a result, silver-poly(methyl methacrylate) nanocomposites were obtained. The dried silver-poly(methyl methacrylate) nanocomposites were dispersed in chloroform and subjected to TEM to observe the silver particle diameter and shape. The result is shown in FIG. 7. As is apparent from the particle distribution of FIG. 7, the silver particles had an average diameter of 6.55±1.27 nm and a uniform particle diameter and shape.

The formation of the silver-poly(methyl methacrylate) nanocomposites was identified by UV/VIS spectrometry. The result is shown in FIG. 8. As shown in FIG. 8, an absorption peak of the nanocomposites appeared at 405 nm.

Comparative Example

Among conventional silver nanoparticles prepared by gamma-rays radiation as in the present invention, silver nanoparticles prepared by using sodium dodecyl sulfate as a stabilizer were reported to have a smallest particle diameter of about 8 nm (*Mater. Lett.*, 1993, 17, 314). In this article, the silver nanoparticles had a considerably wide diameter distribution ranging from 5 nm to 37 nm, having an average particle diameter of 13 nm.

Regarding silver-polymer nanocomposites, silver-poly(butyl acrylate-co-styrene) nanocomposites prepared by gamma-rays irradiation of a water-in-oil

emulsion were reported to have an average particle diameter of 8.5 nm (*Chem. Commun.* 1998, 941). In this article, the particle diameter distribution was not apparent due to low magnification of the TEM photograph.

Experimental Example 1: Stability of silver nanoparticle colloid solution

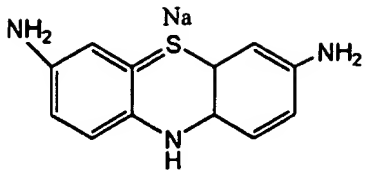
To determine stability of the silver nanoparticle colloid solution prepared in Example 1, the silver nanoparticle colloid solution was left for 10 months at room temperature and observed by TEM. The result is shown in FIG. 9. As shown in FIG. 9, the particle size was slightly increased, but the particle shape and the colloid state were stably maintained without precipitation.

Experimental Example 2: Interaction between silver and polyvinyl pyrrolidone

An Infrared (IR) spectrum was measured for the silver nanoparticle colloid solution prepared in Example 2 to determine whether the silver and the polyvinyl pyrrolidone interact. The result is shown in FIG. 10. In FIG. 10, (a) is the IR spectrum for polyvinyl pyrrolidone alone, and (b) is the IR spectrum for the silver nanoparticles prepared in Example 2 by using the polyvinyl pyrrolidone as a stabilizer. It is evident from the results of FIG. 10 that the silver and the polyvinyl pyrrolidone interact in the colloid solution.

Experimental Example 3: Surface enhanced Raman scattering measurement

Surface enhanced Raman scattering occurs in silver nanoparticle colloid solutions. The Raman scattering spectrum of the silver nanoparticles prepared in Example 2 was measured with respect to pH of a 1.0×10^{-5} M thionin solution. The results are shown in FIG. 11. The results of FIG. 11 show that the silver nanoparticles can be applied to surface enhanced Raman spectroscopy for assaying a trace of organic substances, including bioorganic substances.



Thionin

Experimental Example 4: Antibacterial activity test in textile

Antibacterial activity was measured in a textile soaked with the silver nanoparticle colloid solution prepared in Example 2, according to the method of KS K 0693. The silver nanoparticle colloid solution of Example 2 was diluted with water to 0.5%. 1.0%, and 1.5%, and textiles were immersed in each of the diluted sample solutions. *Staphylococcus aureus* (ATCC 6538) strain was used for the antibacterial activity test. The results for each of the samples are shown in Table 1 below. As shown in Table 1, the silver nanoparticle colloid solution according to the present invention showed a 99.9% antibacterial activity for all colloid dilutes.

Table 1

Sample	Antibacterial Activity (% on average)
0.5%	99.9%
1.0%	99.9%
1.5%	99.9%

In samples containing no silver nanoparticle colloid solution according to the present invention, white spots by the *Staphylococcus aureus* (ATCC 6538) strain were observed, as shown in FIG. 13. In contrast, in the samples containing the silver nanoparticle colloid solution according to the present invention, the *Staphylococcus aureus* (ATCC 6538) strain was hardly observed, as shown in FIG. 12.

Industrial Applicability

According to the present invention, a metal nanoparticle colloid solution

and metal-polymer nancomposites having a uniform particle diameter and shape can be prepared at room temperature on a large scale. Conventional methods using a reducing agent are ineffective to prepare uniform particles on a large scale. As is apparent from the observation by TEM, the metal nanoparticles according to the present invention have a more uniform, smaller particle diameter and shape, compared to metal nanoparticles that have been reported to date, and thus a great surface area to volume ratio. Therefore, the metal nanoparticle colloid solution and metal-polymer nanocomposites according to the present invention have a high level of antibacterial activity even when only a trace is used. The metal nanoparticles according to the present invention have a nano-scaled particle size and are greatly adsorptive due to polymer surrounding individual particles, and thus shows an effect of shielding electromagnetic waves when applied to the field of thin film coating, in addition to antibacterial and sterilizing effects.

What is claimed is:

1. A method for preparing a metal nanoparticle colloid solution, comprising:

dissolving a metal salt and a water-soluble polymer in water, a non-aqueous solvent, or a solvent mixture of water and a non-aqueous solvent;

purging a reaction container containing the solution with nitrogen or argon gas; and

radiating radioactive rays onto the solution.

2. The method of claim 1, further comprising dilution and ultrasonic treatment after radiating the radioactive rays onto the solution.

3. The method of claim 1, wherein the water-soluble polymer includes polyvinyl pyrrolidone, a copolymer having vinyl pyrrolidone as a first polymerization unit, and a fatty acid-substituted or unsubstituted polyoxyethylene.

4. The method of claim 1, wherein the copolymer having vinyl pyrrolidone as the first polymerization unit includes (1-vinyl pyrrolidone)-acrylic acid copolymer, (1-vinyl pyrrolidone)-vinyl acetic acid copolymer, (1-vinyl pyrrolidone)-styrene copolymer, and (1-vinyl pyrrolidone)-vinyl alcohol copolymer.

5. The method of claim 3, wherein the fatty acid-substituted polyoxyethylene includes polyoxyethylene stearate and polyoxyethylene palmitate.

6. The method of claim 1, wherein the metal salt is a salt of at least one metal selected from the group consisting of silver, copper, nickel, palladium, and platinum.

7. The method of claim 6, wherein the metal salt is a silver salt.

8. The method of claim 7, wherein the silver salt includes silver

nitrate, silver perchlorate, silver sulfate, and silver acetate.

9. The method of claim 1, wherein the non-aqueous solvent is an alcoholic solvent.

10. The method of claim 9, wherein the alcoholic solvent is at least one selected from the group consisting of isopropyl alcohol, methanol, ethanol, and ethylene glycol.

11. A metal nanoparticle colloid solution prepared by the method according to any one of claims 1 through 10.

12. The metal nanoparticle colloid solution of claim 11 for use as an antibacterial agent, a sterilizer, a conductive adhesive, conductive ink, or an electromagnetic wave shielder for an image display.

13. A method for preparing metal-polymer nanocomposites, comprising:

dissolving a metal salt and a polymeric stabilizer in a solvent mixture of water and a non-aqueous solvent;

purging a reaction container containing the solution with nitrogen or argon gas; and

radiating radioactive rays onto the solution to obtain precipitates.

14. The method of claim 13, further comprising dilution and ultrasonic treatment after the formation of the precipitates.

15. The method of claim 13, wherein the polymeric stabilizer is at least one polymer selected from the group consisting of polyethylene, polyacrylonitrile, poly(methyl (meth)acrylate), polyurethane, polyacrylamide, and polyethylene glycol.

16. The method of claim 13, wherein a surfactant is added to the solvent mixture of water and the non-aqueous solvent together with the metal salt and the polymeric stabilizer to form an emulsion.

17. The method of claim 16, wherein the surfactant is polyoxyethylene sorbitan mono-oleate.

18. The method of claim 13, wherein the metal salt is a salt of at least one metal selected from the group consisting of silver, copper, nickel, palladium, and platinum.

19. The method of claim 18, wherein the metal salt is a silver salt.

20. The method of claim 19, wherein the silver salt includes silver nitrate, silver perchlorate, silver sulfate, and silver acetate.

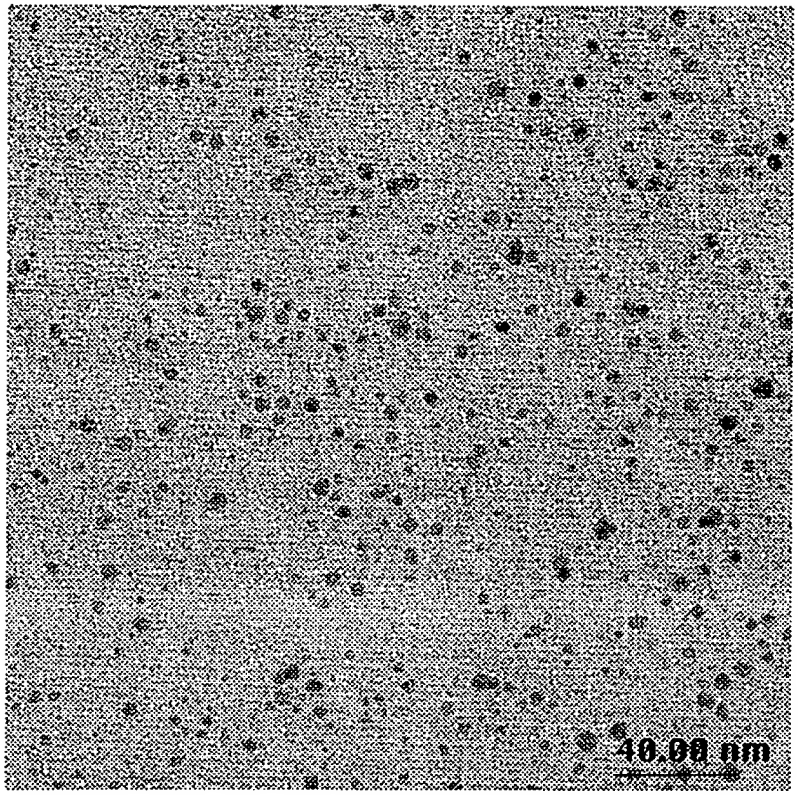
21. The method of claim 13, wherein the non-aqueous solvent is an alcoholic solvent.

22. The method of claim 21, wherein the alcoholic solvent is at least one selected from the group consisting of isopropyl alcohol, methanol, ethanol, and ethylene glycol.

23. Metal-polymer nanocomposites prepared by the method according to any one of claims 13 through 22.

24. The metal-polymer nanocomposites of claim 23 for use as an antibacterial agent, a sterilizer, a conductive adhesive, conductive ink, or an electromagnetic wave shielder for an image display.

Fig. 1


40.00 nm

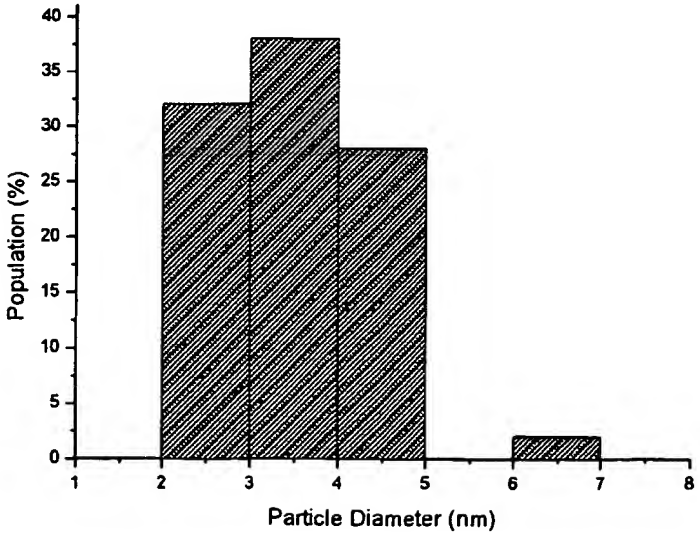

Population (%)
0
5
10
15
20
25
30
35
40
1
2
3
4
5
6
7
8
Particle Diameter (nm)

Fig. 2

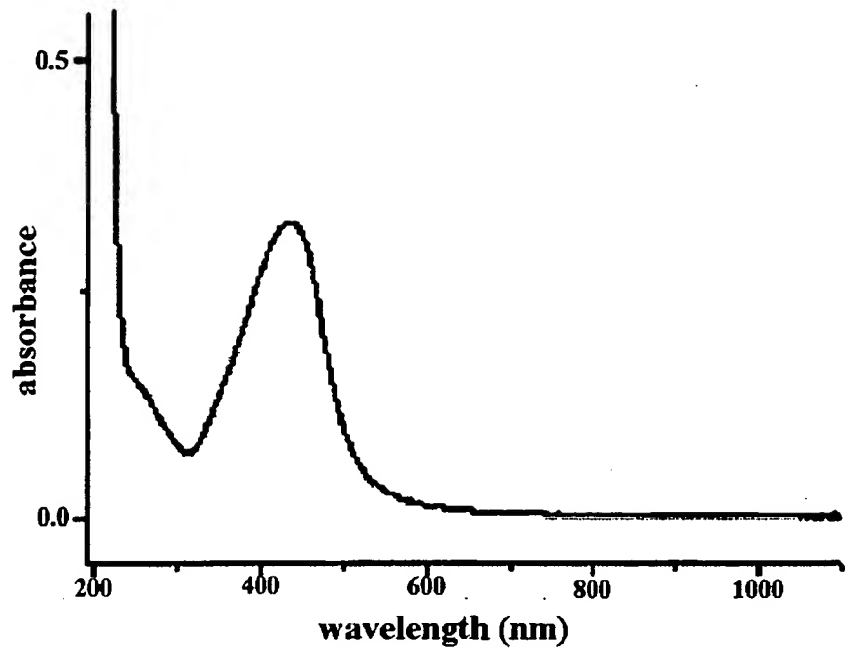

0.5
0.0
absorbance
200
400
600
800
1000
wavelength (nm)

Fig. 3

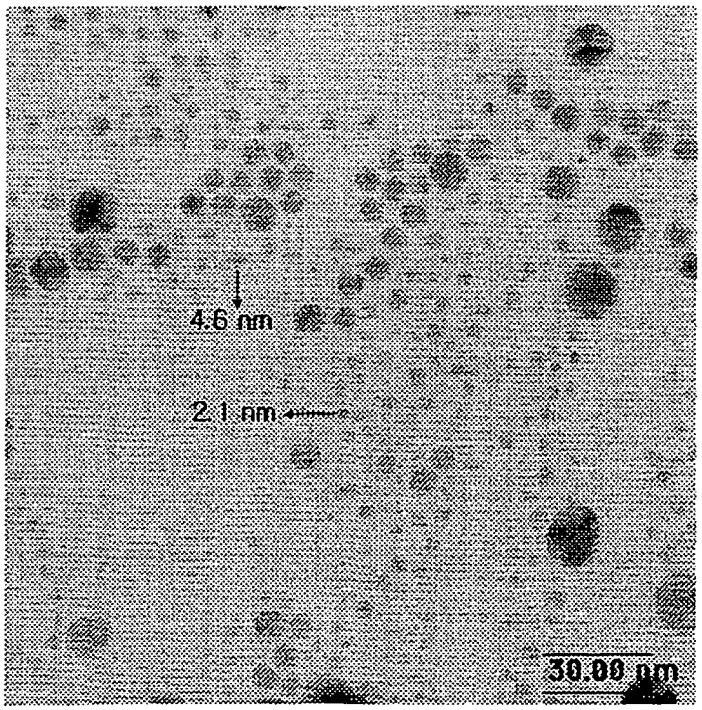
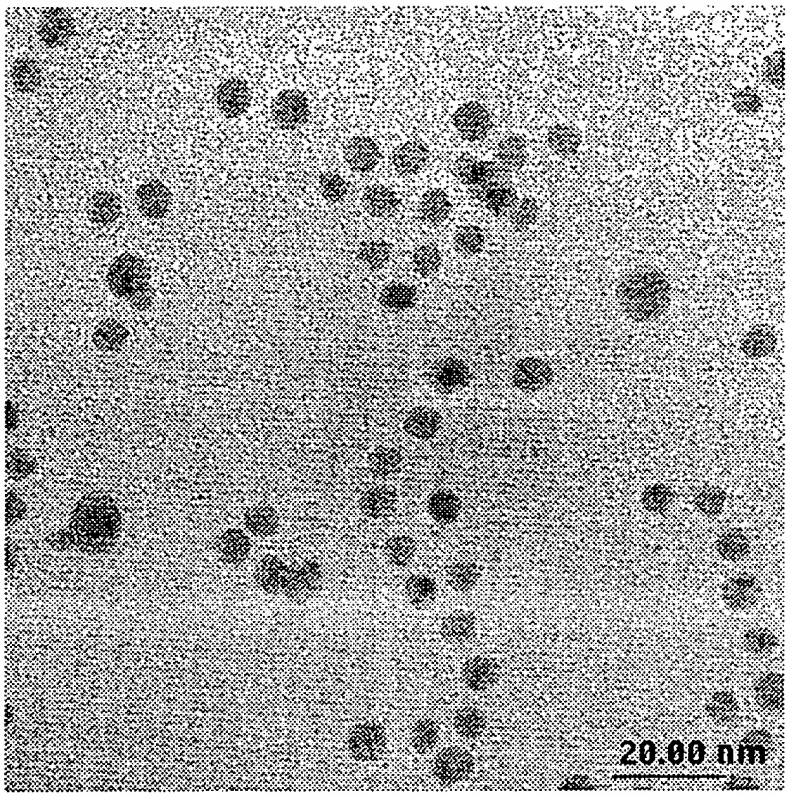

4.6 nm
2.1 nm
30.00 nm

Fig. 4


20.00 nm

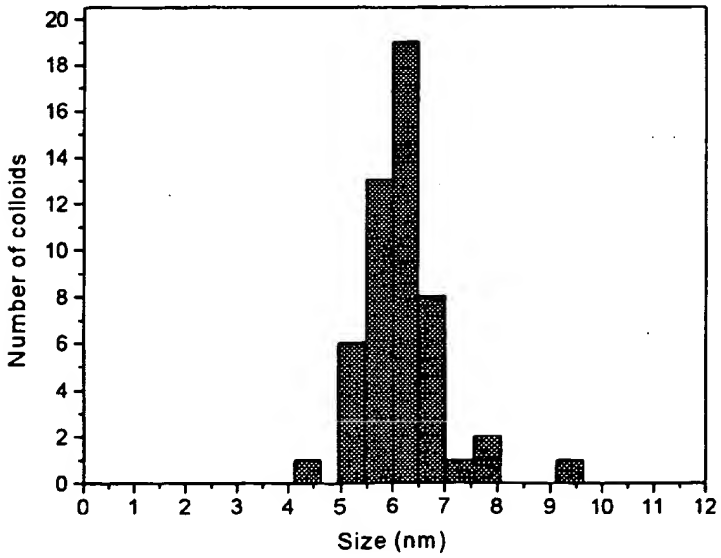

Number of colloids
0
2
4
6
8
10
12
14
16
18
20
0 1 2 3 4 5 6 7 8 9 10 11 12
Size (nm)

Fig. 5

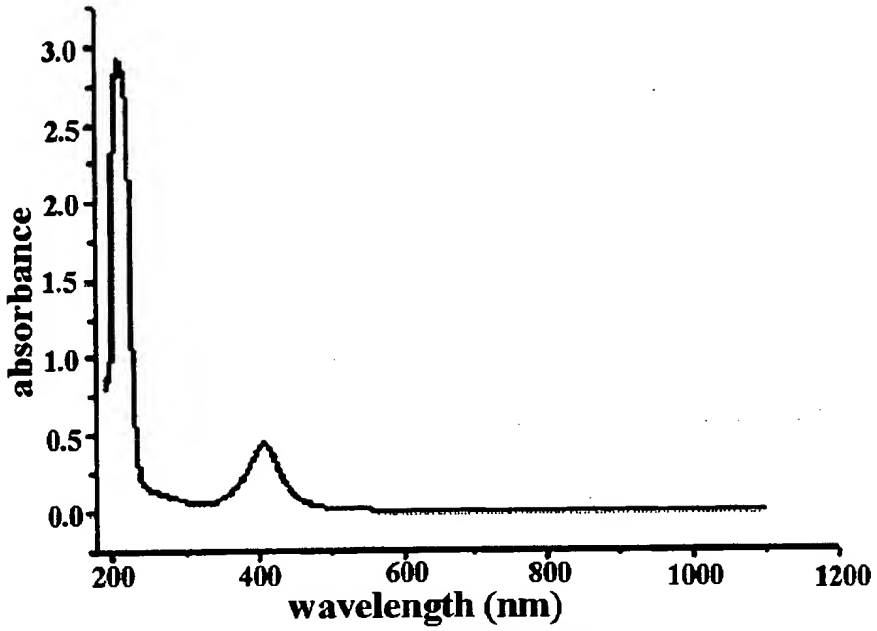

absorbance
3.0
2.5
2.0
1.5
1.0
0.5
0.0
200
400
600
800
1000
1200
wavelength (nm)

Fig. 6

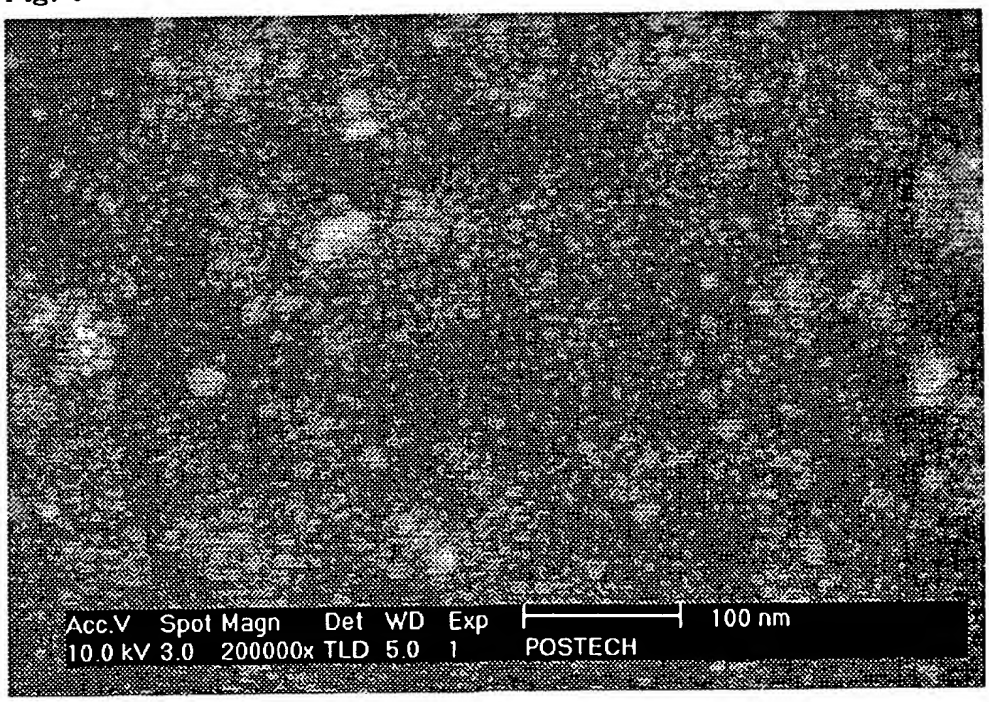
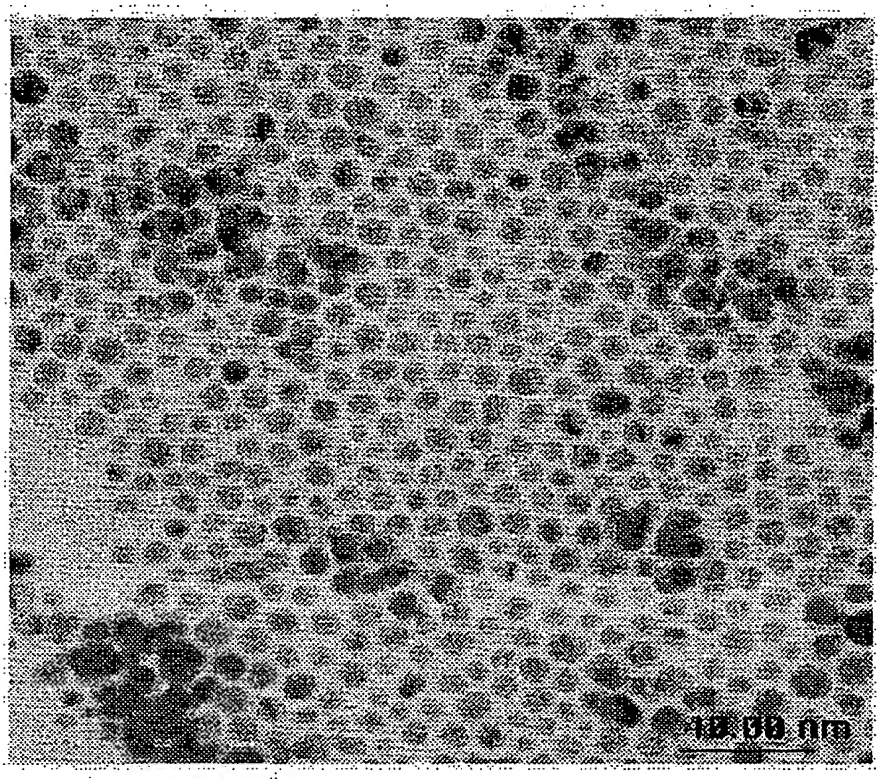

Acc.V Spot Magn Det WD Exp
10.0 kV 3.0 200000x TLD 5.0 1
100 nm
POSTECH

Fig. 7


100.00 nm

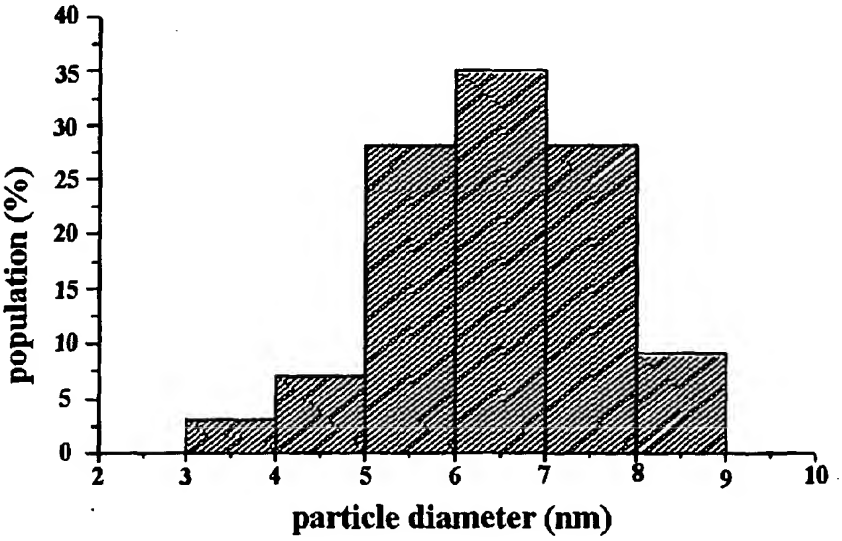

population (%)
0
5
10
15
20
25
30
35
40
2
3
4
5
6
7
8
9
10
particle diameter (nm)

Fig. 8

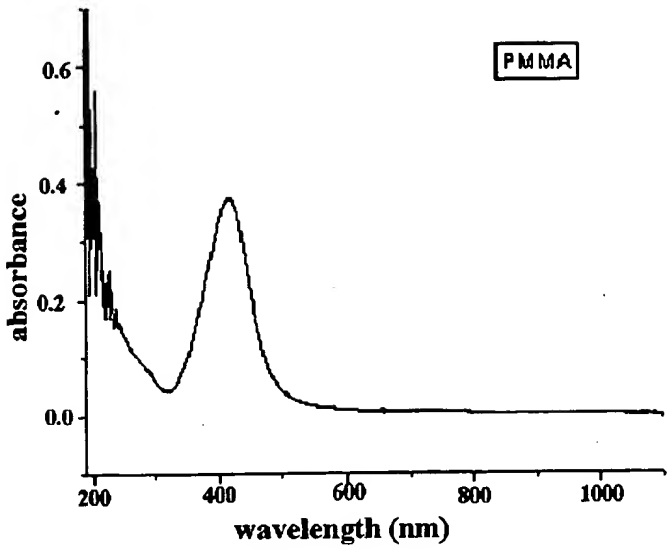

PMMA
absorbance
0.0
0.2
0.4
0.6
200
400
600
800
1000
wavelength (nm)

Fig. 9

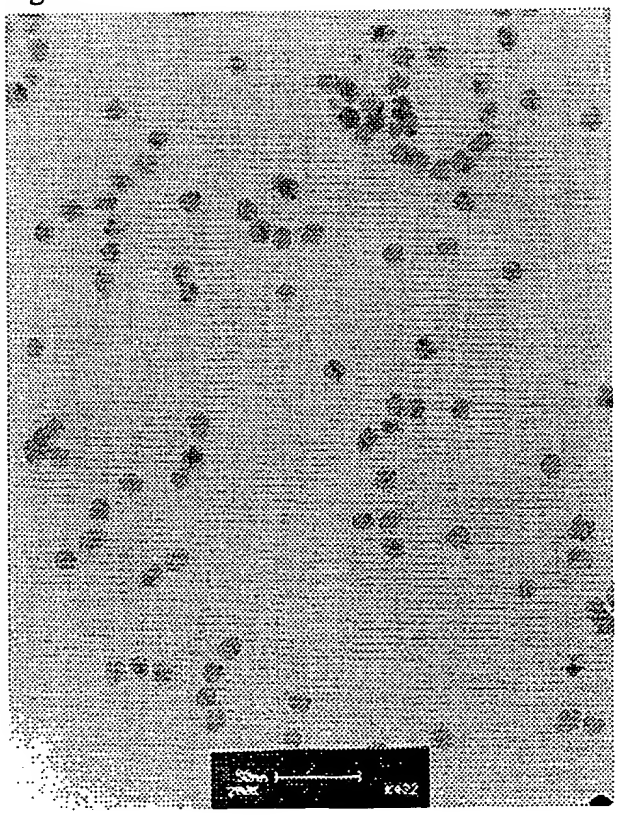


Fig. 10

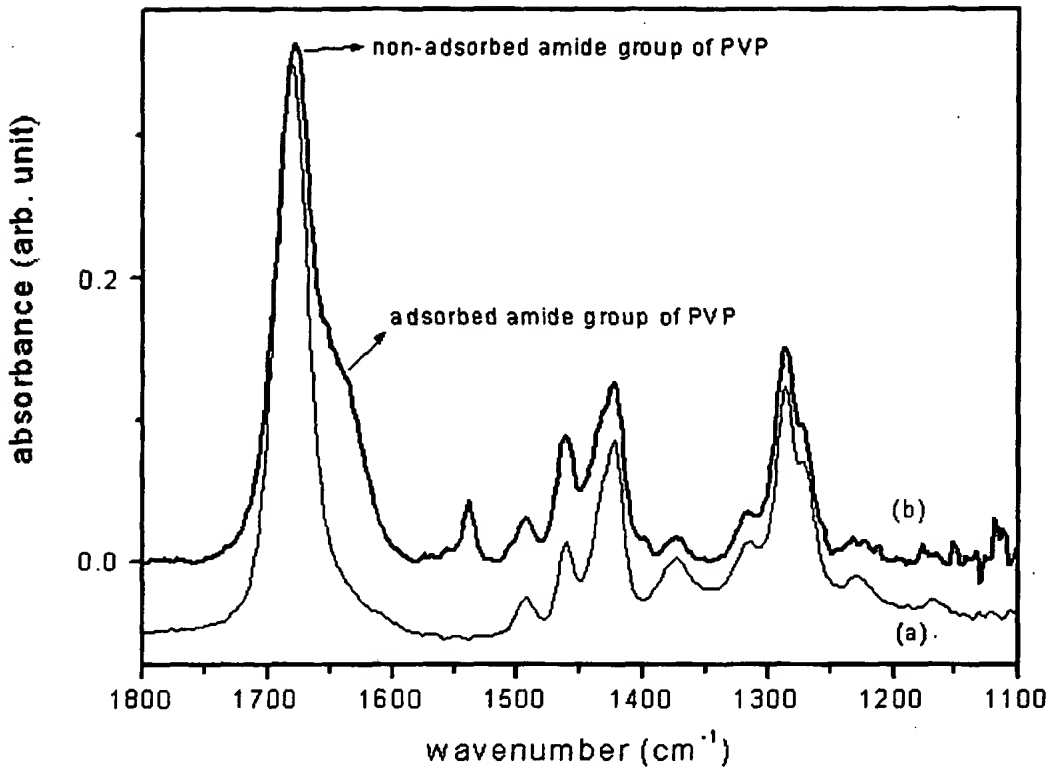

non-adsorbed amide group of PVP
adsorbed amide group of PVP
absorbance (arb. unit)
0.2
0.0
(b)
(a)
1800
1700
1600
1500
1400
1300
1200
1100
wavenumber (cm-1)

Fig. 11

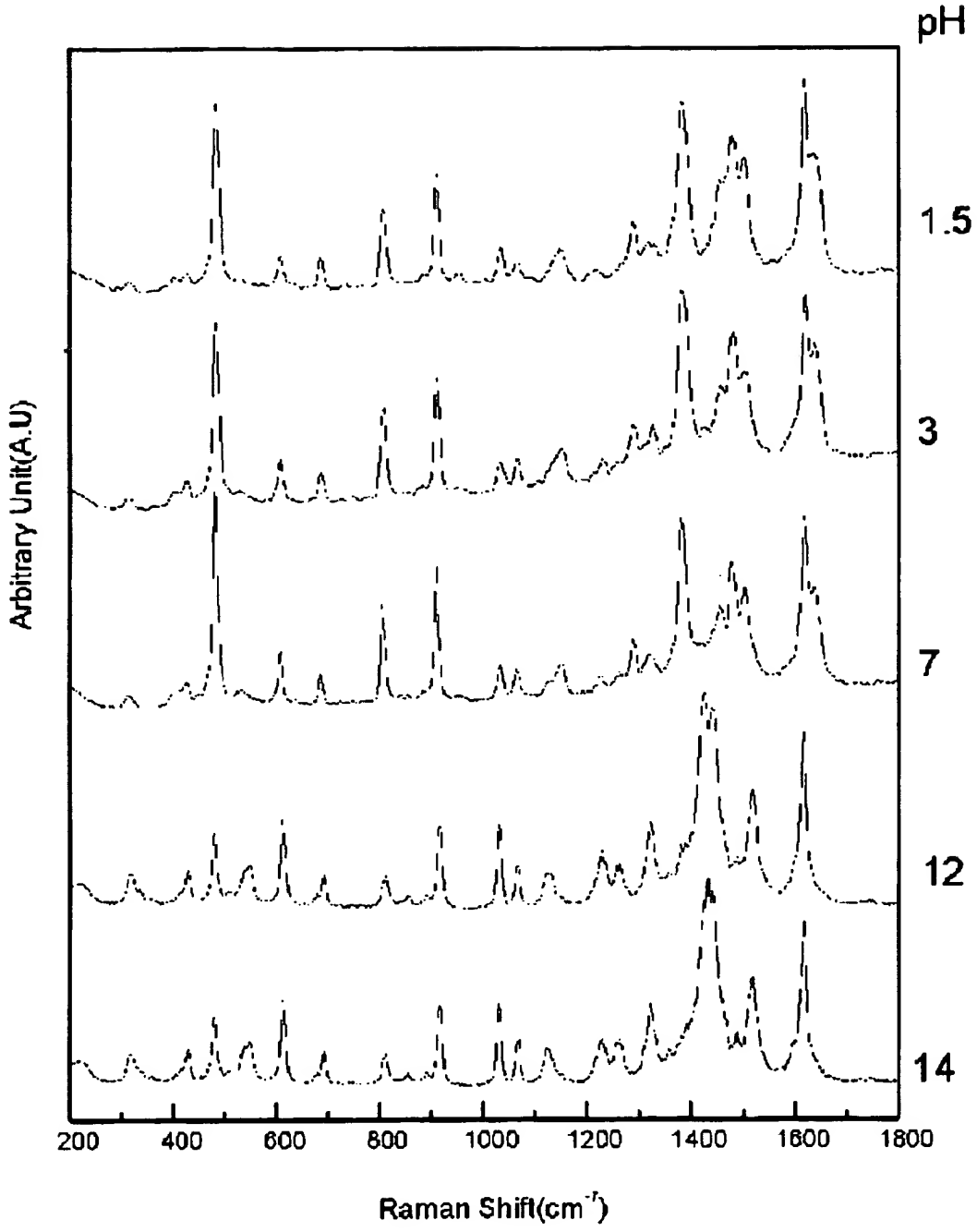

pH
1.5
3
7
12
14
Arbitrary Unit(A.U)
200
400
600
800
1000
1200
1400
1600
1800
Raman Shift(cm-1)

Fig. 12

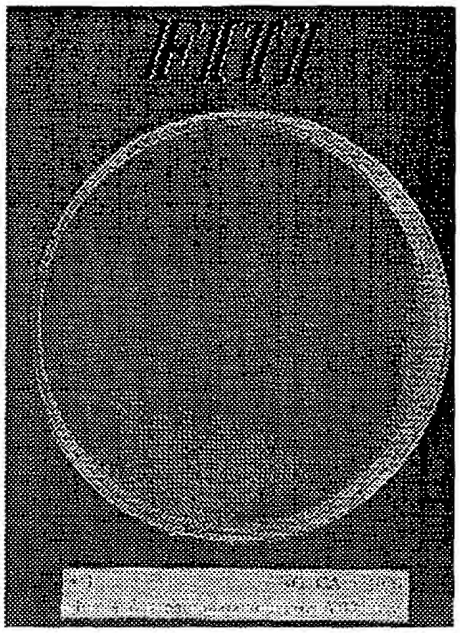
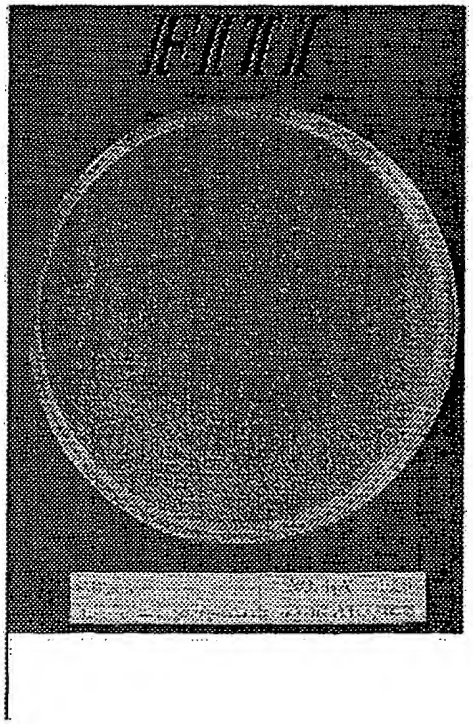


Fig. 13



INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/KR02/00800

A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 B01J 13/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for invention since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NPS, PAJ "metal, polymer, nanoparticle"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E, Y	KR 2002-43363 A (Korea Advanced Institute of Science and Technology) 10 JUNE 2002 See the whole document (especially, see claim 1-11)	1-23
Y	DE19639632 A1 (ZENNECK ULRICH PROF DR) 04 SEP. 1998 See abstract	1, 13
A	US 5,431,967A (The University of Texas) 11 JULY 1995 See the whole document	1-23
A	US 5,912,069A (Sigma Laboratories of Arizona) 15 JUNE 1999 See the whole document	1-23
A	US 5,560,960A (THE UNITED STATES OF AMERICA, NAVY) 1 OCT. 1996 See the whole document	1-23

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevence

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2002-43363 A	10. 06. 2002	NONE	
DE 19639632 A1	04. 09. 1998	NONE	
US 5,431,967A	11. 07. 1995	AU 643700 B2 EP 416852 B1 JP 3183530 A2	25. 11. 1993 16. 07. 1997 09. 08. 1991
US 5,912,069A	15. 06. 1999	NONE	
US 5,560,960A	01. 10. 1996	EP 787044 A1 WO 9614165 A1	06. 08. 1997 17. 05. 1996

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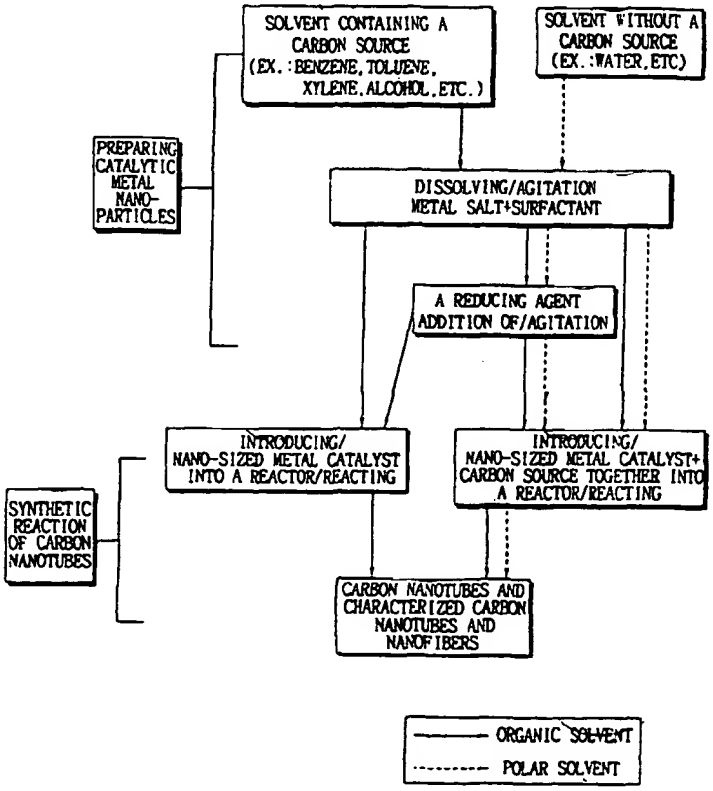
(54) Title: PREPARATION OF CARBON NANOTUBES



(57) Abstract: The present invention is to provide a process for the preparation of carbon nanotubes or nanofibers, which comprises introducing in a gaseous phase a colloidal solution of matal nanoparticles optionally containing a surfactant together with an optional carbon source into a heated reactor, and carbon nanotubes or nanofibers thus prepared. According to the present invention, the shape and structure of carbon nanotubes or nanofibers can be easily controlled, the carbon nanotubes or nanofibers can be continuously produce on a large scale, the apparatus and the process for the preparation of nanotubes or nanofibers are simplified, and carbon nanotubes or nanofibers having various shapes, structures and properties can be easily and cheaply prepared. Further, the process of the present invention is highly reproducible and favorable in industry.

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patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PREPARATION OF CARBON NANOTUBES

Technical Field

The present invention relates to a process for the preparation of carbon nanotubes, particularly to a process enabling continuous bulk preparation of carbon nanotubes or carbon nanofibers in which catalytic metal nanoparticles having preliminarily controlled composition, particle size and particle distribution are continuously introduced. More specifically, the present invention is to provide a process for the preparation of carbon nanotubes or nanofibers, which comprises introducing in a gaseous phase a colloidal solution of metal nanoparticles, preferably containing an optional surfactant, together with a carbon source into a heated reactor, and carbon nanotubes or carbon nanofibers obtained from the same process. Therefore, the present invention is highly reproducible and industrially promising.

Background Art

A carbon nanotube is a substance in which a carbon atom is bonded to neighboring three carbon atoms, these bonded carbon atoms forming a hexagonal ring with other adjacent bonded carbon atoms and such rings being repeated in a

honeycomb pattern to form a sheet which rolls into a cylindrical tube.

Such carbon nanotubes may have a diameter ranging from several angstroms (Å) to several nanometers (nm), with the length ranging from ten-folds to thousand-folds of the diameter. Extensive studies have been carried out on the synthesis of carbon nanotubes since these nanotubes have a morphological feature as described above and excellent thermal, mechanical and electrical characteristics originating from their chemical bonding. It is now expected that utilization of carbon nanotubes having these characteristics would lead to the development of numerous products which still face the technical limitation of the existing materials, and to the impartation of new, previously unpossessed characteristics to developed products.

For the synthesis of carbon nanotubes, various techniques have been proposed, including arc discharge, laser evaporation, thermal chemical vapor deposition (CVD), catalytic synthesis, plasma synthesis and the like [See US Patent 5,424,054 (arc discharge); Chem. Phys. Lett. 243, 1-12(1995) (laser evaporation); Science, 273: 483-487(1996) (laser evaporation); US Patent 6,210,800 (catalytic

synthesis); US Patent 6,221,330 (gaseous phase synthesis); WO 00/26138 (gaseous phase synthesis)]. In these methods, carbon nanotubes are synthesized under severe reaction conditions, for example, at high temperatures of several hundred degrees to several thousand degrees in Celsius, or in vacuum. Further, the type of reaction used is a batch-type reaction, instead of a continuous flow type reaction, such that continuous preparation of carbon nanotubes is impossible, and only small amounts of carbon nanotubes are produced in batch reactions.

Accordingly, said methods have the problem of facing limitation in mass production of nanotubes at low costs, and therefore it is desired to develop a suitable process for the gas-phase synthesis, especially a process for continuous synthesis which is industrially useful.

The Oakridge National Laboratory and R. E. Smalley et al of Rice University in the United States reported respectively processes for the synthesis of carbon nanotubes in the gaseous phase. In these gas-phase synthetic processes, an organometallic compound in which a transition metal is bound to an organic compound in the molecular level, such as ferrocene or iron pentacarbonyl, is introduced in the solid state into a reactor as a

catalyst promoting the synthesis of carbon nanotubes. As shown in the above-mentioned prior art, the conventional processes for the gas-phase synthesis of carbon nanotubes are carried out in a reactor that is divided into two reaction zones. A catalytic metal precursor is first introduced in the solid state to the first reaction zone where the precursor is vaporized in the molecular level by gradual heating. The vaporized catalytic metal molecules are transferred to the second reaction zone which is maintained at a higher temperature, where the molecules are subjected to pyrolysis so that the metal atoms form ultrafine particles. These ultrafine particles aggregate, while colliding into each other, to form fine particles, and then the fine metal particles may be used as the catalyst for the growth of carbon nanotubes. However, it has been reported that the particles are required to have a certain size, preferably of nanometers, in order to function as a catalyst [See US Patent 6,221,330 or WO 00/26138].

However, in the conventional gaseous synthesis methods for carbon nanotubes, catalyst particles form irregularly in the reactor, and thus it is practically impossible to expect uniform growth of catalyst particles in controlled

size. Moreover, as transition metals differ from each other in their physical properties, it is difficult to prepare nanometer-sized catalyst particles comprising two or more transition metal species and having uniform composition and controlled size. Consequently, it is extremely difficult or hardly possible to produce characterized carbon nanotubes comprising two or more transition metals in uniform composition. Furthermore, since it is impossible to control the particle size and metal composition of catalytic metals in the conventional gas-phase synthetic processes, it is difficult to produce carbon nanotubes of high purity. In particular, the process suggested by Smalley et al. has a drawback that the reaction should be carried out at elevated pressures.

The inventors of the present invention have discovered that carbon nanotubes can be produced by suspending nanometer-sized fine metal particles in a gaseous phase as a metal catalyst which has the greatest influence on the properties of carbon nanotubes produced and simultaneously supplying a carbon source, and that most of the problems faced by the conventional gas-phase synthetic processes as described above can be overcome by this novel method.

According to the present invention, (a) since the

particle size and composition of the catalyst are pre-determined, the shape and structure of the carbon nanotubes produced thereby can be more easily controlled; (b) since the catalyst as well as the carbon source can be supplied continuously, continuous mass production of carbon nanotubes is possible; (c) since the carbon source is supplied together with the catalytic metal nanoparticles, the process itself can be simplified; and (d) since the reaction process is carried out in mild conditions, the carbon nanotubes or nanofibers having a variety of shapes, structures and properties can be prepared easily at reasonable costs. Conclusively, the process of the present invention is industrially very promising.

Disclosure of Invention

Therefore, the present invention is to provide a process for the preparation of carbon nanotubes or nanofibers, characterized in that nanoparticles of elemental metals or metal compounds (hereinafter, referred to as "metal nanoparticles") or a colloidal solution thereof are introduced in a gaseous phase into a reactor together with an optional carbon source, and to provide carbon nanotubes

or nanofibers thus prepared.

According to a preferred embodiment of the present invention, metal nanoparticles are prepared in the form of a colloidal solution optionally containing a surfactant and then are introduced in a gaseous phase into a reactor.

More specifically, the process of the present invention consists of the following steps:

(1) preparing a colloidal solution containing metal nanoparticles in the presence or absence of a surfactant,

(2) introducing the resulting colloidal solution into a heated reactor together with a carrier and/or a carbon source, either separately or in the form of a gaseous mixture, and

(3) forming carbon nanotubes or nanofibers therefrom.

According to a more preferred embodiment, metal nanoparticles or a colloidal solution thereof may be introduced together with or separately from a carbon source, but preferably they are introduced in the form of a mixture for the formation of uniform carbon nanotubes.

In the present invention, "introducing nanoparticles or a colloidal solution thereof in a gaseous phase" means suspending the fine nanometer-sized particles in a gaseous

phase by spraying, injection or atomization, that is, forming a gaseous colloid. Although the nanoparticles of the present invention may be used in the powder form, it is more advantageous to use them in the form of a colloidal solution for the uniformity in the amount of supply, homogeneity in the mixing with the carrier and/or the carbon source, and feasibility of conversion into a gaseous phase.

In general, a colloid represents a solution of solid particles whose size ranges from 1000 Da (Dalton, a unit for the molecular weight) to 0.45 μm (or 0.2 μm). However, "a colloidal solution" used herein means a solution comprising particles of a few nanometers to a few hundred nanometers in size, and occasionally precursors thereof as well.

In the present invention, the term "nanoparticles of elemental metals or metal compounds" or "metal nanoparticles" means nanoparticles having an average particle size in the order of nanometers, for example, of a few nanometers to a few hundred nanometers, in which the metals exist in the elemental and/or compound forms. It also means nanoparticles including those liquid particles having a size in the above-described range, which are

obtained by dissolving or dispersing elemental metals or metal compounds in a solvent (e.g., sol particles), or the particles of an emulsion or a dispersion.

The metals in the metal nanoparticles of the present invention may be present in the form of elements, inorganic or organic compounds, or mixtures thereof, and may consist of a single metal species or of two or more species, such as in the form of an alloy or a composite.

Hereinbelow, the invention will be explained in more detail.

When the particle size is in the order of nanometers (typically 300 nm or less), the particles may be different from those of larger sizes in their properties and performance. Nanometer-sized particles have increased surface area per unit mass, and subsequently show improved performance and altered properties such that the melting point of the particles decreases and the color or the particles varies with the size.

Further, the fine particles of nanometer size may be present in the state of being suspended in a gaseous phase and have high reactivity. The present inventors have done studies to develop a method a way of using such nanometer-sized fine metal particles as a catalyst suitable for the

synthesis of carbon nanotubes, particularly for a process for the preparation of carbon nanotubes in a gaseous phase. To the inventors' knowledge, none of the methods that have been proposed thus far make use of fine metal particles prepared in advance, which are introduced in a gaseous phase into the gas-phase synthesis of carbon nanotubes.

In the present invention, the metal nanoparticles or a colloidal solution thereof may be prepared by techniques such as mechanical grinding, co-precipitation, spraying, sol-gel processing, electrolysis, emulsion processing, inverse emulsion processing or the like.

For example, US Patent 5,238,625 discloses a process for preparing a zirconia sol consisting of tetragonal crystal particles of 0.5 μm or less in size by sol-gel processing. US Patent 5,911,965 discloses a process for preparing a solution or sol of stable oxide polytungstate containing about 17% of tungsten oxide from a solution of acidified tungsten oxide precursor by sol-gel processing. This patented invention does not mention about the particle size or distribution of the sol particles, but the particle size is presumed to be of nanometer level. US Patent 6,107,241 discloses a process for preparing by sol-gel processing an amorphous titanium peroxide sol having a particle size of 8

to 20 nm and a sol concentration of 1.40 to 1.60% which is capable of long-term storage at room temperature. US Patent 6,183,658 discloses a process for preparing nanometer-sized, non-cohesive iron-containing oxide particles having a uniform particle size distribution, the surface of which is modified with a silane compound to prevent cohesion. The above-listed patents are incorporated into the present invention for reference.

In particular, US Patent 5,147,841 discloses a process for preparing a colloidal solution of elemental metal nanoparticles by adding a metal salt to an organic solvent containing a surfactant to form homogeneous inverse micelle particles and reducing the metal salt within the micelle particles. This patent is incorporated into the present invention for reference.

The particle size of colloidal metal particles prepared according to the emulsion processing or inverse emulsion processing method are generally of the order of several nanometers to several hundred nanometers and may be adjusted in accordance with the reaction conditions. A surfactant is added to form homogeneous micelle particles and to prevent cohesion of the colloidal metal particles.

As described above, the emulsion processing or inverse

emulsion processing method is further advantageous in that metal particles containing two or more metal species may be prepared in the form of a composite or an alloy, and that the weight, the particle size and its distribution of the metal salt micelles may be easily controlled by the types and amounts used of the surfactant and the solvent. The metal salt micelles having a controlled particle size and its distribution can be used as a catalyst either per se or after being reduced to elemental metal particles without substantial changes in the particle size and its distribution. This means that the composition, particle size and distribution of the metal nanoparticles used as a catalyst can be controlled.

Hence, in a preferred embodiment of the present invention, the metal salt micelle particles produced as described above are used as a catalyst either per se or in a reduced form. Specifically, the present invention provides a process for preparing carbon nanotubes, which comprises the following steps :

(1a) preparing a colloidal solution containing metal salt nanoparticles by adding metal salts to a solvent, such as water, or a polar or nonpolar organic solvent, containing a surfactant,

(1b) optionally reducing the metal salt nanoparticles in the colloidal solution,

(2) introducing the resulting colloidal solution into a heated reactor together with a carrier and/or a carbon source, either separately or in the form of a gaseous mixture, and

(3) forming carbon nanotubes or nanofibers therefrom.

The metals used in the present invention are not particularly limited, and may be any metals that can be simply added or used as a catalyst in the process for preparing carbon nanotubes or nanofibers. Examples of such metals include transition metals such as iron, cobalt or nickel; noble metals such as platinum or palladium; and alkali and alkaline earth metals. The types of the metal compounds which may be used in the present invention are not particularly limited, and examples include the elemental metals listed above, their oxides, nitrides, borides, fluorides, bromides and sulfides, and mixtures thereof. If necessary, a metal which does not act as a catalyst in the process of the present invention may be added with a catalytic metal in the form of an alloy or a mixture, and this does not depart from the spirit and the scope of the invention.

Meanwhile, according to the present invention, the colloidal solution of metal nanoparticles may exist as a gaseous colloid for some time when the droplets containing the metal particles are made to suspend in a gas, since these particles are nanometer-sized fine particles. The methods to transform the colloidal solution into a gaseous phase and to suspend the droplets in a gas are not particularly limited, and conventional methods in the art, for example, direct spraying, siphon spraying, atomization, etc., may be used.

The droplets of the colloidal solution containing metal nanoparticles introduced into a reactor in a gaseous phase, as described above, are immediately transformed into nanometer-sized metal particles owing to the high temperature in the reactor and may be used as a catalyst for the growth of carbon nanotubes.

In a variation of the present invention, even if a colloidal solution containing nanoparticles of metal compounds such as oxides is introduced into the reactor without preliminary reduction, the particles are reduced into elemental metal particles in a short time because the fineness of the particles increases their reactivity; or unreduced particles may also be used per se in the process

of synthesizing carbon nanotubes.

In the present invention, the surfactant forms fine micelle particles with the metal nanoparticles in the solvent, facilitates uniform distribution of the metal particles, and maintains the size of the metal particles by preventing the cohesion of the metal particles until they are introduced into the reactor. The surfactant may be nonionic, cationic, anionic or zwitterionic, and all types of surfactant may be used, for example, hydrocarbons, silicone compounds, fluorocarbon compounds and so on. The amount of the surfactant used in the invention is not particularly limited and may be adequately selected by a person having ordinary skill in the pertinent art.

Reduction of the metal salts may also be carried out by adding at least one reducing agent selected from the group consisting of, for example, inorganic compounds such as hydrazine, $LiBH_4$ and $NaBH_4$; surfactants having a functional group with reducing power such as ethylene oxide; and organic compounds with reducing power. Reduction may proceed to the extent that the metal salts are reduced partially or completely to metals.

Solvents which may be used to prepare a colloidal solution include water, and polar or nonpolar organic

solvents. A polar or nonpolar organic solvent may be selected from the group consisting of aromatic hydrocarbons such as benzene, toluene and xylene; aliphatic organic solvents such as hexane, heptane and octane; polar solvents such as ethanol and propanol; and mixture thereof.

In the present invention, the metal nanoparticles or the colloidal solution thereof may be introduced into the reactor together with a carrier. As the carrier, mention may be made of inert gases such as argon, neon, helium and nitrogen; and polar or nonpolar solvents aforementioned.

Together with the gaseous mixture of the colloidal solution and an optional carrier or separately, a gaseous or liquid carbon source may be supplied. As the carbon source, said surfactants and organic solvents may be used as they are, and other organic compounds selected from the group consisting of hydrocarbons such as carbon monoxide, saturated or unsaturated aliphatic hydrocarbons having 1 to 6 carbon atoms and aromatic hydrocarbons having 6 to 10 carbon atoms may be used as well. Such a carbon source may have 1 to 3 heteroatoms selected from the group consisting of oxygen, nitrogen, chlorine, fluorine and sulfur.

Since the surfactant and/or the solvent of the colloidal solution may also act as the carbon source, when the

contents thereof are high, additional carbon sources may not be needed.

According to a preferred embodiment of the present invention, a characterized gas such as H_2, H_2S or NH_3 may be supplied together with the carbon source.

The process of the present invention may be carried out in a reactor used for reactions such as thermal heating, chemical vapor deposition (CVD), plasma heating, radio frequency (RF) heating and so on. However, the type of the reactor is not limited as long as carbon nanotubes may be produced therein. The reaction processes to form carbon nanotubes or nanofibers using such reactors are described in the prior art above-mentioned. Therefore, without being particularly limited in the present invention, the process parameters for carrying out the present invention, such as the temperature, time and pressure, may be easily decided by a person having ordinary skill in the art from said prior art.

Meanwhile, in the processes of prior art for producing carbon nanotubes in a gaseous phase in which metal particles are formed by aggregation of metal atoms and used as catalysts, it is reported that the lower reaction temperature results in the smaller particle size of the

catalytic metal, and subsequently in the smaller length and diameter of the carbon nanotubes produced. However, as the particle size of the catalytic metal is adjusted before being introduced into the reactor in the process of the present invention, it is possible to control the length and diameter of the carbon nanotubes substantially irrespective of the reaction temperature.

The process of the present invention may be favorably applied to the syntheses of carbon nanotubes having various structures and morphologies as well as tube-typed nano-scale structures such as graphite nanofibers (GNF), since the catalyst of the present invention may comprise two or more metal species in any compositions.

Brief Description of Drawings

Figure 1 is a schematic flow diagram showing briefly the process for synthesizing carbon nanotubes of the present invention.

Figures 2 to 6 are the scanning electron micrographs (SEM) or transmission electron micrographs (TEM) of the carbon nanotubes prepared in Example 1 (Figure 2), Example 3 (Figure 3), Example 5 (Figure 4), Example 9 (Figure 5) and Example 13 (Figure 6), respectively.

Figures 7 to 9 are the scanning electron micrographs (SEM) or transmission electron micrographs (TEM) of the carbon nanotubes prepared by using a metal mixture in Example 26.

Figures 10 and 11 are the scanning electron micrograph and transmission electron micrograph of the graphite nanofibers (GNF) synthesized in Example 27.

Figure 12 is the transmission electron micrograph of the graphite nanofibers synthesized in Example 28.

Best Mode of Carrying Out the Invention

The present invention will be understood more easily with reference to the following examples. However, these examples are intended to illustrate the invention and are not to be construed to limit the scope of the invention.

Example 1

To 40 ml of benzene, 3.516 g (10 % by weight of benzene) of polyoxyethylene(20) sorbitan monolaurate (Tween®-20) and 0.0648 g (a quantity required for preparing a 0.01M benzene solution) of $FeCl_3$ were added, and the mixture was stirred for 24 hours to give a solution of nanoparticles. Tween®-20 is a nonionic surfactant which plays a role of stabilizing the nanoparticles to be formed and reducing the metal ions.

It was confirmed by transmission electron microscopy (TEM) that the nanoparticle solution obtained above contained fine metal particles with a size ranging from 2 to 20 nm.

Reaction was carried out by introducing the obtained solution (0.34 ml/min) together with a carrier gas (Ar, flow rate: 100 sccm) into a reactor at 800℃ for 20 minutes. The product was obtained as a black powder.

The product obtained above was analyzed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). It was confirmed that carbon nanotubes of a mean diameter of about 60 nm were obtained, and the SEM micrograph showing the nanotubes is shown in Figure 2.

Example 2

To a solution of nanoparticles prepared in the same manner as in Example 1, 0.01 g (0.005 mol) of $LiBH_4$ was added as a reducing agent, and the mixture was stirred for 24 hours to give a solution of nanoparticles with a size ranging from 2 to 20 nm. Somewhat severe flocculation of the particles was observed, compared with the case where no reducing agent was added.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, and carbon

nanotubes with a mean diameter of about 70 nm were obtained.

Example 3

Carbon nanotubes with a mean diameter of about 60 nm were obtained in the same manner as in Example 1, except that toluene or xylene was used respectively in place of benzene. The SEM micrograph of the carbon nanotubes is shown in Figure 3.

Example 4

A result analogous to that of Example 2 was obtained by performing the procedure in the same manner as in Example 2, except that toluene or xylene was used respectively in place of benzene.

Example 5

To 40 ml of benzene, 3.516 g (10 % by weight of benzene) of cetyl trimethyl-ammoniumbromide (CTAB) and 0.0648 g (a quantity required for preparing a 0.01M benzene solution) of $FeCl_3$ were added, and the mixture was stirred for 24 hours. CTAB is a cationic surfactant which plays a role of stabilizing the nanoparticles to be formed. 0.01 g (0.005M) of $LiBH_4$ was added as a reducing agent to the above-obtained solution, and the mixture was stirred for 24 hours to give a solution of nanoparticles with a size ranging from 2 to 20 nm.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, and carbon nanotubes with a mean diameter of about 70 nm were obtained. The SEM micrograph of the carbon nanotubes is shown in Figure 4.

Example 6

A result identical with that of Example 5 was obtained by performing the procedure in the same manner as in Example 5, except that toluene or xylene was used respectively in place of benzene.

Example 7

A solution of nanoparticles with a size ranging from 2 to 20 nm was obtained in the same manner as in Example 5, except that an anionic surfactant, sodium dodecyl-sulfate (SDS), was used.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, and carbon nanotubes with a mean diameter of about 70 nm were obtained.

Example 8

A result identical with that of Example 7 was obtained by performing the procedure in the same manner as in Example 7, except that toluene or xylene was used respectively in place of benzene.

Example 9

A solution of nanoparticles with a size ranging from 2 to 50 nm was obtained in the same manner as in Example 1, except that water was used in place of benzene as the solvent.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, but this time together with a carbon source (ethylene gas, 50 sccm), and carbon nanotubes with a mean diameter of about 60 nm were obtained. The SEM micrograph of the carbon nanotubes is shown in Figure 5.

Example 10

A result identical with that of Example 9 was obtained by performing the procedure in the same manner as in Example 9 except that methane gas was used in place of ethylene as the carbon source.

Examples 11 and 12

Results identical with those of Examples 9 and 10 were obtained by performing in the same manner as in Examples 9 and 10, except that ethanol was used in place of water.

Example 13

To a solution of nanoparticles prepared in the same manner as in Example 9, 0.065 g (0.005 mol) of hydrazine

was added as a reducing agent, and the mixture was stirred for 24 hours to give a solution of nanoparticles with a size ranging from 2 to 50 nm. Somewhat severe flocculation of the particles was observed, compared with the case where no reducing agent was added.

The solution obtained above was introduced into the reactor in the same manner as in Example 9, and carbon nanotubes with a mean diameter of about 80 nm were obtained. The SEM micrograph of the nanotubes is shown in Figure 6.

Example 14

A result identical with that of Example 9 was obtained by performing in the same manner as in Example 9, except that methane gas was used in place of ethylene as the carbon source.

Example 15

To a solution of nanoparticles prepared in the same manner as in Example 11, 0.065 g (0.005 mol) of hydrazine was added as a reducing, and the mixture was stirred for 24 hours to give a solution of nanoparticles with a size ranging from 2 to 50 nm. Somewhat severe flocculation of the particles was observed, compared with the case where no reducing agent was added.

The solution obtained above was introduced into the

reactor in the same manner as in Example 11, and carbon nanotubes with a mean diameter of about 70 nm were obtained.

Example 16

A result identical with that of Example 15 was obtained by performing in the same manner as in Example 15, except that methane gas was used in place of ethylene as the carbon source.

Example 17

A solution of nanoparticles with a size ranging from 2 to 50 nm was obtained in the same manner as in Example 5, except that water was used as the solvent in place of benzene and 0.0065 g (0.005 mol) of hydrazine was used as the reducing agent in place of 0.01 g (0.005 mol) of $LiBH_4$.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, but this time together with a carbon source (ethylene gas, 50 sccm) and with the reactor heated to 900°C. Carbon nanotubes of a mean diameter of about 70 nm were obtained.

Example 18

A result identical with that of Example 17 was obtained by performing the procedure in the same manner as in Example 17, except that methane gas was used in place of ethylene.

Examples 19 and 20

Results identical with those of Examples 17 and 18 were obtained by performing in the same manner as in Examples 17 and 18, except that ethanol was used in place of water.

Example 21

A solution of nanoparticles with a size ranging from 2 to 50 nm was obtained in the same manner as in Example 7, except that water was used as the solvent in place of benzene and 0.0065 g (0.005 mol) of hydrazine was used as the reducing agent in place of 0.01 g (0.005 mol) of $LiBH_4$.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, but this time together with a carbon source (ethylene gas, 50 sccm). Carbon nanotubes with a mean diameter of about 70 nm were obtained.

Example 22

A result identical with that of Example 21 was obtained by performing the procedure in the same manner as in Example 21, except that methane gas was used in place of ethylene as the carbon source.

Example 23

A result identical with Example 21 was obtained by performing in the same manner as in Example 21, except that

ethanol was used in place of water.

Example 24

A result identical with Example 22 was obtained by performing in the same manner as in Example 22, except that ethanol was used in place of water.

Example 25

Results analogous to those of Examples 1 to 8 were obtained by repeating the procedures of Examples 1 to 8, except that the experiments were carried out in a globe box, which is isolated from the outside contact to prevent the formation of metal oxides, in order to produce pure fine metal particles.

Described below is an example of synthesizing carbon nanotubes by means of catalytic nanoparticles comprising two metal species.

Example 26

This example illustrates the result obtained when a catalyst was prepared in which a metal selected Pt, Pd, Rh, Ir, Ru, and Ni known for their high activity in dehydrogenation of hydrocarbons that are used as the carbon source, and iron together form nanoparticles, and was used in the synthesis of nanotubes.

To 40 ml of benzene, 3.516 g (10 % by weight of benzene)

of Tween®-20 and 0.0648 g (a quantity required for preparing a 0.01M benzene solution) of $FeCl_3$ were added, and the mixture was stirred for 2 hours. Then, H_2PtCl_6, $PdCl_2$, H_2IrCl_6, $RuCl_3$ or $NiCl_2$ was added to said mixture in an amount such that the atomic ratio of iron : metal was 7 : 3, and the mixture was stirred for another 24 hours to give a solution of nanoparticles.

It was confirmed by transmission electron microscopy (TEM) that the above-obtained nanoparticle solution contained fine metal particles with a size ranging from 4 to 30 nm. The size of the alloy catalyst particles appeared slightly greater than that of the particles of pure iron obtained in other cases. However, the size of the nanoparticles did not vary much with the type of metal.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, and carbon nanotubes with a mean diameter of about 60 nm were obtained. It was recognized from Figures 7, 8 and 9 that the arrangement of carbon atoms was more regular in this case, compared with the nanotubes produced with a catalyst of iron only.

Specifically, Figure 7 shows the result of the synthesis of nanotubes using the catalyst made of an alloy of iron

and nickel, and it shows that nanotubes were produced uniformly in a large quantity. Figure 8 shows the result of the synthesis of nanotubes using a catalyst made of an alloy of iron and platinum, and it shows that the arrangement was more regular, compared with the case where a catalyst of iron only was used, and that apparently the byproduct of carbon black was not produced. Figure 9 shows the result of the synthesis of nanotubes using a catalyst made of an alloy of iron and palladium.

Example 27

This example illustrates the result obtained when nanometer-sized catalyst particles comprising iron and copper were prepared and used in the synthesis of nanotubes in order to synthesize graphite nanofibers (GNF) which are utilized in the media for the storage of hydrogen.

To 40 ml of benzene, 3.516 g (10 % by weight of benzene) of Tween®-20 and 0.0648 g (a quantity required for preparing a 0.01M benzene solution) of $FeCl_3$ were added, and the mixture was stirred for 24 hours. Then, $CuCl_2$ was added to said mixture in an amount such that the atomic ratio of iron : copper is 3 : 1, and the mixture was stirred for another 24 hours to give a solution of nanoparticles with a size ranging from 4 to 30 nm.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, and GNF with a mean diameter of about 100 nm were obtained. The SEM and TEM micrographs thereof are shown in Figures 10 and 11.

Example 28

In this example, the same process was repeated under different reaction conditions using the nano-sized catalyst particles prepared in Example 1, in order to synthesize graphite nanofibers (GNF) which are utilized in the media for the storage of hydrogen.

Reaction was carried out by introducing the above-obtained solution (0.34 ml/min) and H_2S gas (10 sccm) into a reactor at 800 °C, together with a carrier gas (Ar, flow rate: 100 sccm) for 20 minutes, and the product was obtained as a black powder. The product obtained was analyzed by scanning electron microscopy (SEM) and a transmission electron microscopy (TEM) to find that GNF with a mean diameter of about 60 nm were obtained, the TEM micrograph of which is shown in Figure 12.

Example 29

This example illustrates the result obtained when nano-sized catalyst particles comprising iron and atomic sulfur were prepared and used in the synthesis of nanotubes in

order to synthesize graphite nanofibers (GNF) which are utilized in the media for the storage of hydrogen.

To 40 ml of benzene, 3.516 g (10 % by weight of benzene) of Tween®-20 and 0.0648 g (a quantity required for preparing a 0.01M benzene solution) of $FeCl_3$ were added, and the mixture was stirred for 2 hours. Then, Na_2S was added to said mixture in an amount such that the atomic ratio of iron : sulfur was 1 : 2, and the mixture was stirred for another 24 hours to give a solution of nanoparticles with a size ranging from 4 to 30 nm.

The solution obtained above was introduced into the reactor in the same manner as in Example 1, and GNF with a mean diameter of about 100 nm were obtained.

Industrial Applicability

In conclusion, according to the present invention, since the particle size and the composition (the types and proportions of the metals) of the metal catalyst introduced are adjusted in advance, the morphology and structure of the carbon nanotubes produced may be more easily controlled. Further, as the metal catalyst can be supplied continuously, carbon nanotubes may be produced continuously in large scales, and the supply of the metal catalyst together with

a carbon source from the outside allows simplification of the process as well as the apparatus. Also, since the reaction conditions are mild, carbon nanotubes or graphite nanofibers having various morphologies, structures and characteristics may be produced easily at reasonable costs. Hence, the process of the present invention is highly reproducible and industrially promising.

What is claimed is :

1. A process for the preparation of carbon nanotubes, which comprises:

(1) preparing a colloidal solution containing metal nanoparticles in the presence or absence of a surfactant,

(2) introducing the resulting colloidal solution into a heated reactor together with a carrier and/or a carbon source either separately or in the form of a gaseous mixture, and

(3) forming carbon nanotubes or nanofibers therefrom.

2. The process according to claim 1, which comprises:

(1a) preparing a colloidal solution containing metal salt nanoparticles by adding a metal salt to a solvent selected from water, or polar or nonpolar organic solvents, which contains a surfactant,

(1b) optionally reducing the metal salt nanoparticles in the colloidal solution,

(2) introducing the resulting colloidal solution into a heated reactor together with a carrier and/or a carbon source either separately or in the form of a gaseous mixture, and

(3) forming carbon nanotubes or nanofibers therefrom.

3. The process according to claim 1, wherein the surfactant

is selected from the group consisting of nonionic, cationic, anionic and zwitterionic surfactants of hydrocarbons, silicones and fluorocarbons.

4. The process according to claim 2, wherein the reducing agent is selected from the group consisting of inorganic compounds such as hydrazine, $LiBH_4$ and $NaBH_4$; surfactants having a functional group with reducing power such as ethylene oxide; organic compounds with reducing power; and mixtures thereof.

5. The process according to claim 1, wherein the polar or non-polar organic solvent is selected from the group consisting of aromatic hydrocarbons such as benzene, toluene and xylene; aliphatic hydrocarbons such as hexane, heptane and octane; alcohols such as ethanol and propanol; water; and mixtures thereof.

6. The process according to claim 1, wherein the metal used is at least one metal selected from the group consisting of transition metals, noble metals, alkali metals and alkaline earth metals.

7. The process according to claim 1, wherein the metal of the metal nanoparticles is selected from the group consisting of elemental metals, their oxides, nitrides, borides, fluorides, bromides and sulfides, and mixtures

thereof.

8. The process according to claim 1, wherein the resulting colloidal solution is introduced continuously into the reactor for continuous production of carbon nanotubes or nanofibers.

9. The process according to claim 1, wherein the gaseous carbon source is selected from the group consisting of said surfactants, said solvents, carbon monoxide, saturated or unsaturated aliphatic hydrocarbons having 1 to 6 carbon atoms, and aromatic hydrocarbons having 6 to 10 carbon atoms, which may have 1 to 3 heteroatoms selected from the group consisting of oxygen, nitrogen, chlorine, fluorine and sulfur.

10. The process according to claim 9, wherein a characterized gas such as H_2, H_2S and NH_3 is additionally supplied.

11. The process according to claim 1, wherein said reactor is a reactor used for reactions such as thermal heating, chemical vapor deposition (CVD), plasma heating and radio frequency (RF) heating.

12. The process according to claim 1, wherein the metal nanoparticles or the colloidal solution thereof is prepared by a method selected from the group consisting of

mechanical grinding, co-precipitation, spraying, sol-gel processing, electrolysis, emulsion processing and inverse emulsion processing.

13. Carbon nanotubes or nanofibers prepared by the process according to any of claims 1 to 12.

FIG.1

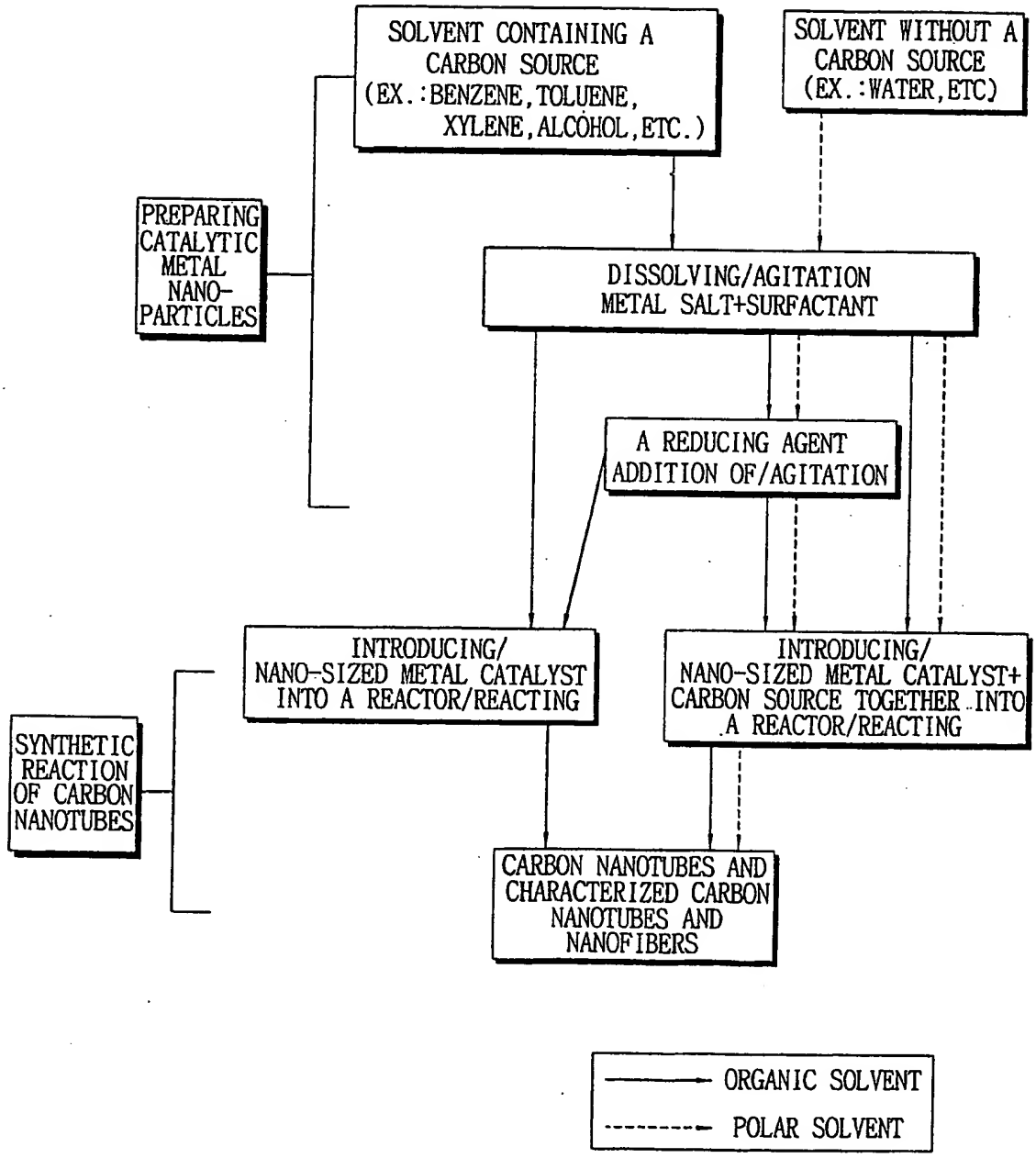

SOLVENT CONTAINING A CARBON SOURCE (EX.:BENZENE,TOLUENE, XYLENE,ALCOHOL,ETC.)
SOLVENT WITHOUT A CARBON SOURCE (EX.:WATER,ETC)
PREPARING CATALYTIC METAL NANO-PARTICLES
DISSOLVING/AGITATION METAL SALT+SURFACTANT
A REDUCING AGENT ADDITION OF/AGITATION
INTRODUCING/ NANO-SIZED METAL CATALYST INTO A REACTOR/REACTING
INTRODUCING/ NANO-SIZED METAL CATALYST+ CARBON SOURCE TOGETHER INTO A REACTOR/REACTING
SYNTHETIC REACTION OF CARBON NANOTUBES
CARBON NANOTUBES AND CHARACTERIZED CARBON NANOTUBES AND NANOFIBERS
ORGANIC SOLVENT
POLAR SOLVENT

FIGURE 2

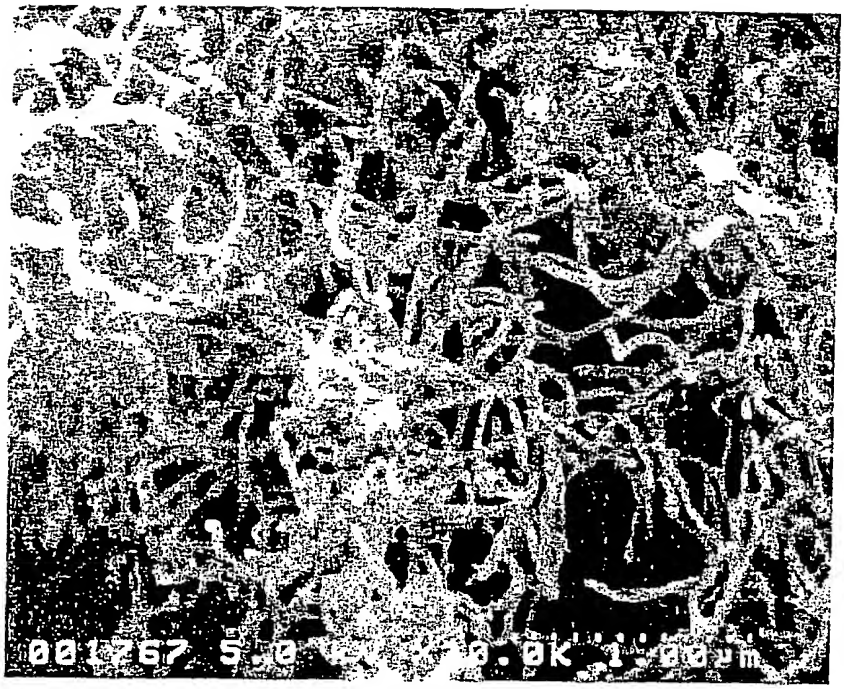


FIGURE 3

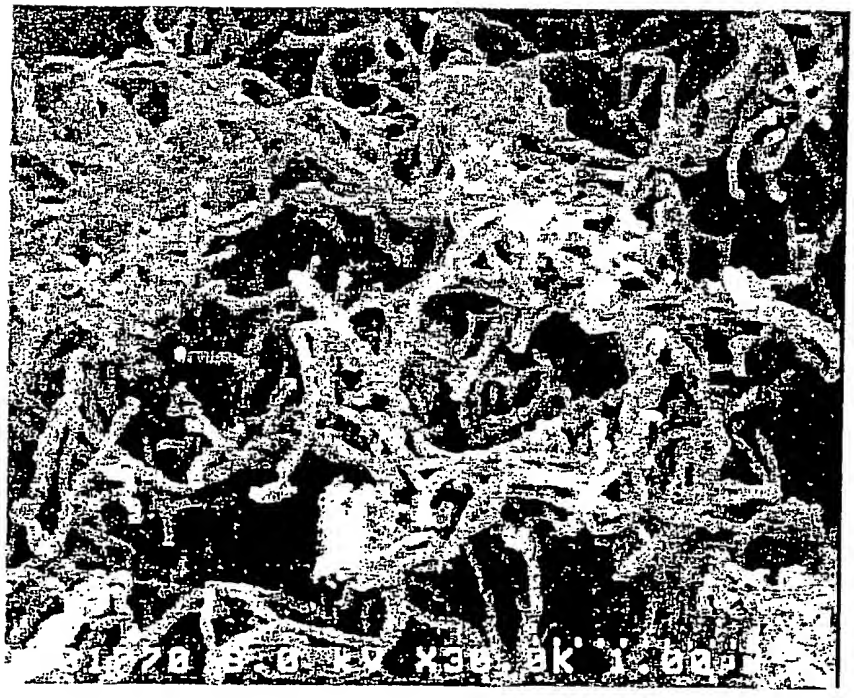


FIGURE 4

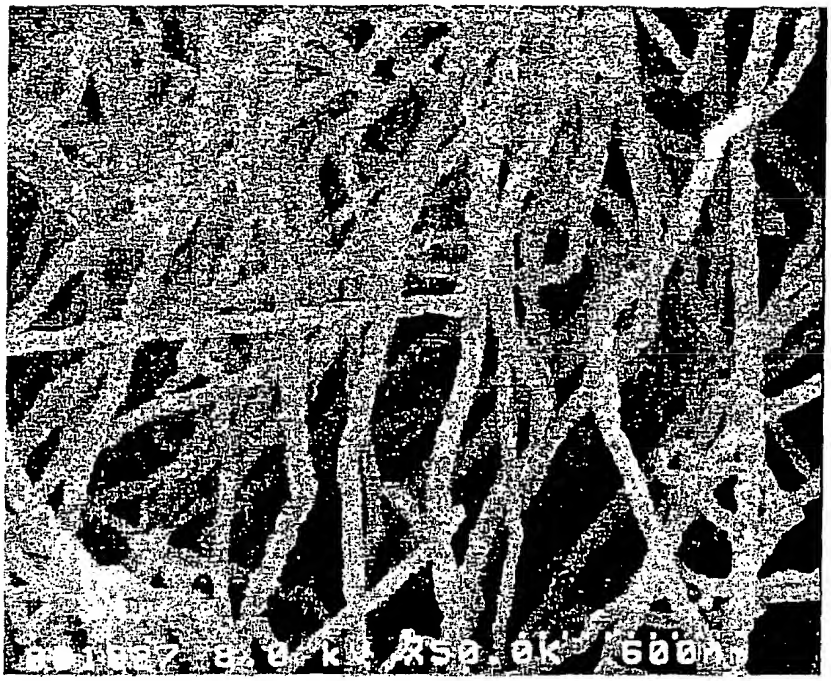


FIGURE 5

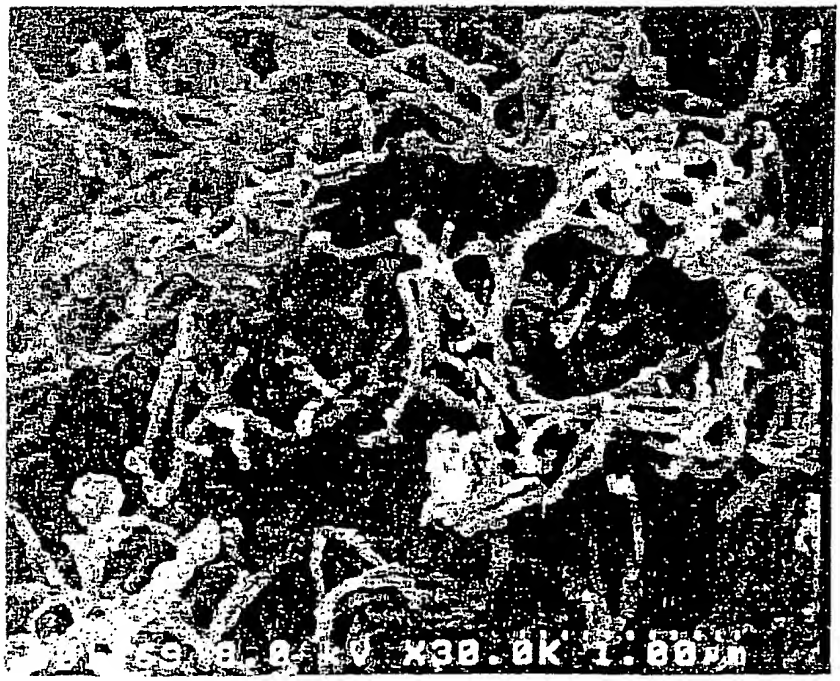


FIGURE 6

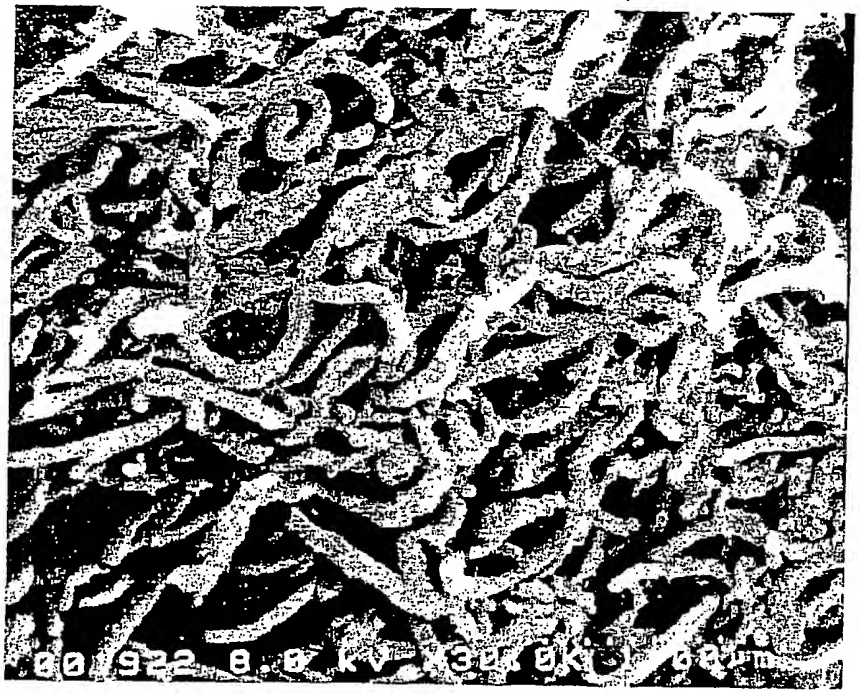


FIGURE 7

FIGURE 8

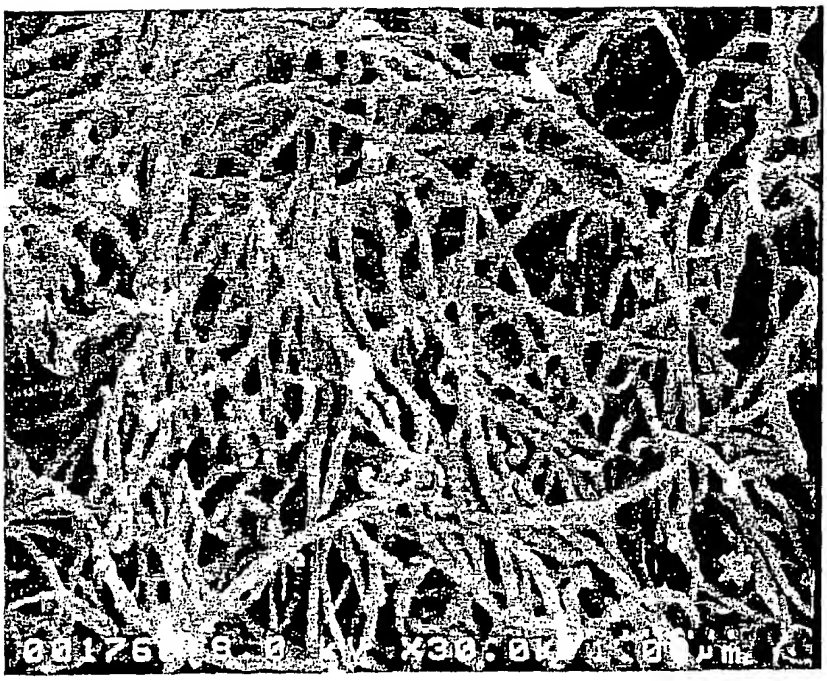


FIGURE 9

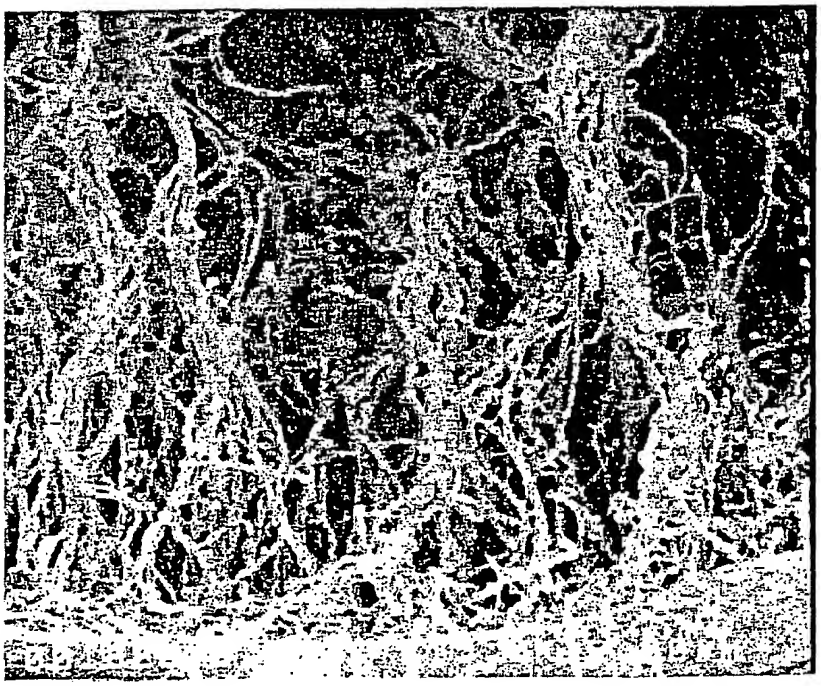


FIGURE 10

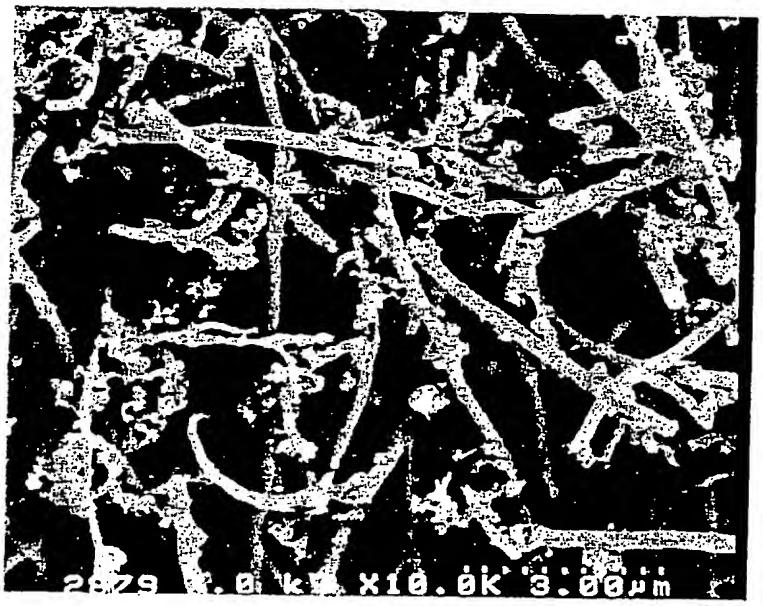


FIGURE 11

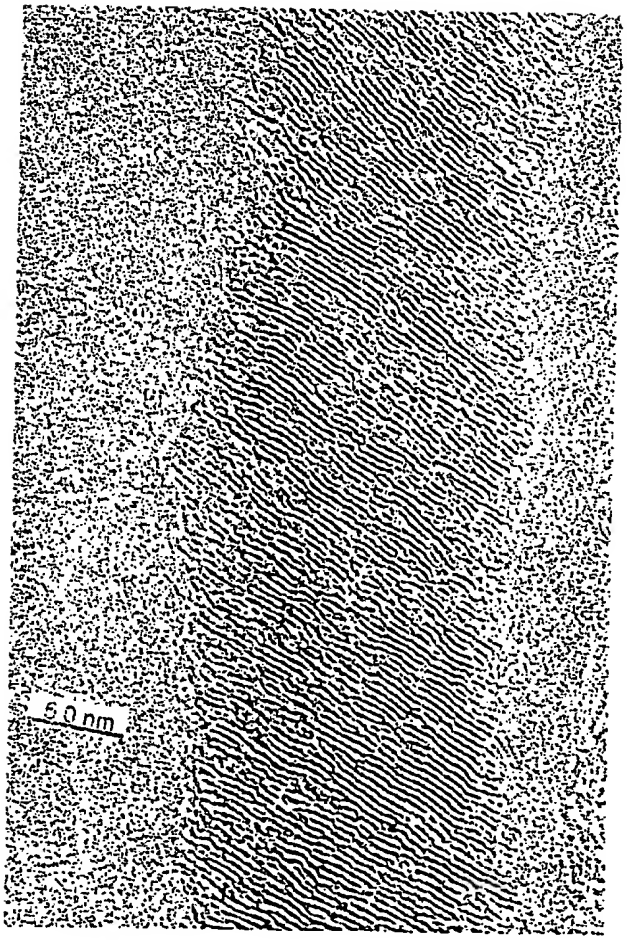
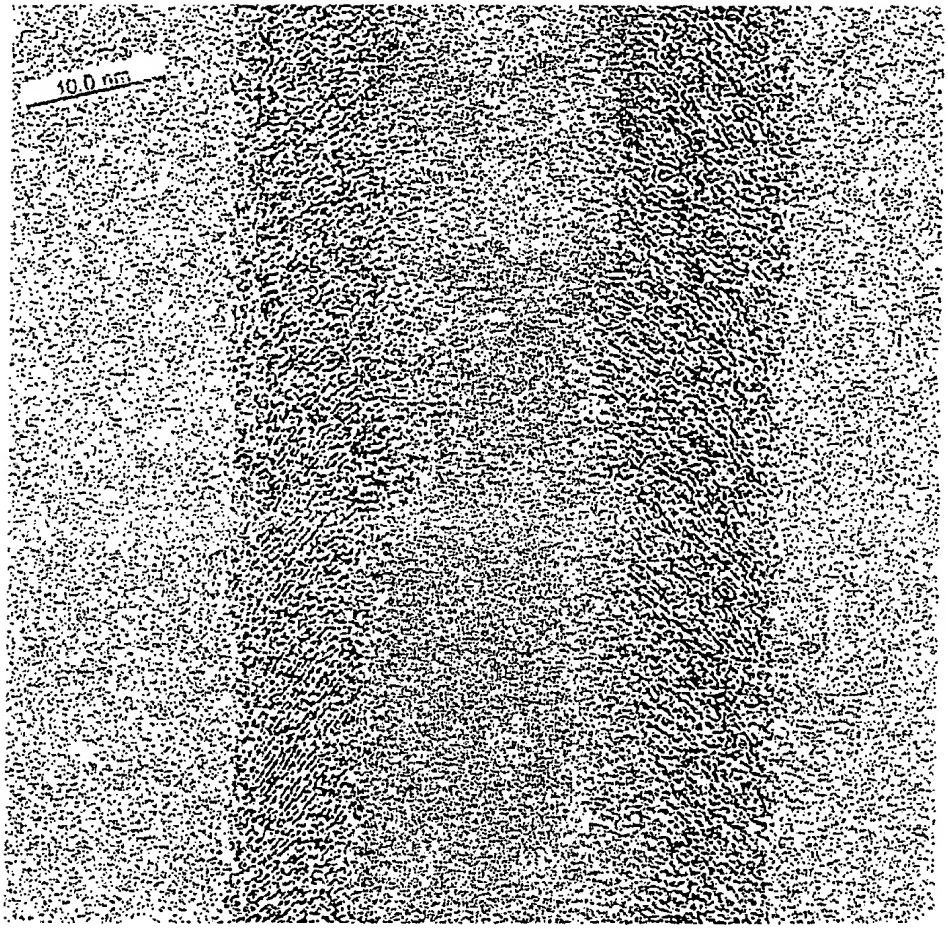


FIGURE 12



INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C01B 31/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C01B 31/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975, Korean Utility models and applications for Utility models since 1975
Japanese Utility models and applications for Utility models since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

NPS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6063243 A (THE REGENTS OF THE UNIV. OF CALIFORNIA) 16. MAY 2000 See The Abstract	1,2
A	US 5424054 A (INTERNATIONAL BUSINESS MACHINES CORP.) cited in the application 13. JUN 1995 See The Claims 1,2,3	1,2

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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WO 02/060553 A1

(54) Title: ISOLATION OF NANOPARTICLES

(57) Abstract: The present invention relates to a process for isolation of nanoparticles from a solution, comprising the steps of providing a solution comprising the nanoparticles; adsorbing the nanoparticles to adsorbing groups on a superporous matrix; and optionally washing the column with a suitable solution; wherein at least the adsorption step is run under dynamic conditions. The nanoparticles isolated in the present process are e.g. plasmids or virus. The present process is especially suitable for large-scale isolation.

ISOLATION OF NANOPARTICLES

Technical field

The present invention relates to a process for isolation of nanoparticles from undesired impurities in a solution. More specifically, the present process is based on adsorption of such nanoparticles to an especially advantageous matrix material.

Background

Conventional bioseparation deals with the isolation of a wide range of biomolecules including small molecules, such as penicillin, up to large molecules, such as proteins. Normally, the size of these particles is below about 10 nm. Recently, as a result of the last year's progress in the fields of genomics and proteomics, an increased interest has been shown in separation of molecules and molecular aggregates of considerably larger size, such as plasmids, viruses etc., in general denoted nanoparticles.

However, most of the hitherto available bioseparation methods have been developed for the processing of molecules of the above-mentioned smaller size. Typically, the target substance is then a protein of an essentially spherical geometry and with a surface similar among the target proteins throughout the population. Such proteins are of a reasonably good diffusivity and many effective carrier materials have been suggested, which rely on the diffusion of protein into a gel structure to binding groups, such as ion exchanging groups or other adsorptive or affinity groups. Thus, most such methods are based on a similarity of target molecules, which principle greatly facilitates the separation procedure since it is then easy to select a distinguishing property and separate accordingly.

However, separation of nanoparticles entails a whole new complex of problems where the prior art methods are only partially applicable. Such problems arise e.g. because of the size of the particles, which leads to a slow diffusion. The diffusivity in free solution is inversely proportional to the size of the particles. Consequently, many nanoparticles will diffuse 10-100 times slower than a normal size protein, resulting in a slow equilibration between the different phases in a separation system.

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Further, in general, nanoparticles will exhibit complex surface structures of a non-uniform nature. In addition to the purely molecular structures, such as the folding of a polypeptide chain, with nanoparticles, there will also be an effect of supermolecular structures and the arrangement of molecular aggregates. Such effects may be shielding or enhancing with respect to the separation performance, but will in any case entail problems due to the difficulties in predicting possible outcomes. One particularly problematic situation arises when the macro-structure is not uniform among the particles in a population, such as in plasmid purification. The same plasmid, i.e. plasmids having the same chemical identity, will normally exist in different conformations, and transformation between said conformations will not occur freely. Thus, if a conventional separation method is applied e.g. on plasmids, the result may well be a division of the population into chemically uniform subfractions, which in turn may result in various complications.

Finally, large aggregates or particles will also give rise to multiple interactions, resulting in an extremely strong binding thereof to a carrier material. Further, such multiple interactions may change with time resulting in an even stronger binding thereof, since it will gradually transform into other conformations enabling additional possibilities to bind.

PerSeptive BioSystems (Biochemica no. 1, 1996) suggests a method of purification of plasmid DNA by anion perfusion chromatography on a matrix comprising through-pores. More specifically, part of the chromatographic flow passes through each individual particle improving its mass transport properties. However, all separation methods using such flow through particles will include an inherent limitation as regards the flow rate, since for an efficient separation to take place, the molecules must be allowed a sufficient time to diffuse into the smaller pores, the size of which are in a range of about 0.15 μm. Accordingly, there is still a need within this field of an even more efficient and flexible method for isolation of nanoparticles, such as plasmids.

Gel permeation chromatography has been suggested for the purification of infectious retroviruses and other enveloped viruses. However, the relative efficiency of such separations is poorly documented in the literature. In particular, Braas et al. (Bioseparation 6:211-228, 1996: Strategies for the isolation and purification of retroviral vectors for gene therapy) discuss how it is difficult to envisage a uniform passage of viral particles and other similarly-sized assemblies, with a typical size of 50-200 nm, in the extra-particle void volume of a packed bed of a gel permeation matrix typically possessing a particle diameter of 60-150 μm. Use of macroporous particles is discussed, but it is suggested that a better strategy may in fact be to consider the custom-design of solid, non-porous particles for the recovery of virions and elimination of impurities. Thus, there is a need of improved methods also for the isolation of viral particles, which need grows stronger with the progress of studies aimed at treating human illness by gene therapeutic methods.

WO 97/19347 relates to a chromatographic separation method and a device comprising a medium with fast kinetics, with high efficiency, with good mechanical properties and with low back pressures. The method and device are suitable for separation of large biomolecules, exemplified as proteins, peptides, nucleic acids, oligonucleotides, cells or viruses. More specifically, the improvement provided by WO 97/19347 is related to the macroporous cross-liked organic polymer, which is prepared by the so-called HIPE (High Internal Phase Emulsion) technique. This technique results in a material with a very open and regular three dimensional structure, wherein an open pore foamlike structure is built up by cavities in the form of spheres with connecting pores between so that a continuous void or pore phase is formed throughout the matrix. However, because of the high porosity of these beads, the available pore surface area and thus the number of binding sites is rather limited. Accordingly, low capacities can be expected.

USP 4,699,717 discloses a process for chromatographic separation of nucleic acid using surface modified carrier materials that similar to the above mentioned WO specification contain cavities, which in the experimental part of this patent specification are

of sizes up to 0.4 μm. The physical appearance of such cavities is illustrated in Fig 1 thereof, from which it appears clearly that the cavity is of a depth which is essentially corresponding to, or slightly superior to, the diameter of said cavity. Thus, cavities are of a different geometry than pores, the depth of which usually exceeds their diameter several times. In any case, these cavities would be too small for nanoparticles, such as large nucleic acids e.g. plasmids. Thus, the example given with plasmids should involve plasmid bound to the outer surface, since the cavities are too small to allow any appreciable entrance of plasmids.

USP 6,143,548 discloses a method of purification of active adenovirus and AAV, which method has been improved compared to the prior art in order not to damage the virus. The design considerations taught relate to the objective of minimising or eliminating damage to the virus by contact with various chromatographic materials. In particular, the approaches are said to be intended to obviate the effect of openings or pores in such materials. Thus, the chromatographic materials suggested comprise pores of a size of up to about 1.2 μm, which is sufficient for the largest known spheroidal viruses. To fulfil the above-mentioned objective, the process is also a batch-type technique, since by this method the virus particles are less likely to enter the pores in the beads where they can become damaged.

Similarly, WO 01/07597 also discloses a chromatographic process, which is run with batch conditions.

Gustavsson et al (Journal of Chromatography A, Volume 734 (1996) 231-241) discloses a superporous agarose, presented as a new material for chromatography. The characterising feature of this agarose is that it comprises both ordinary diffusion pores and very wide pores, known as superpores or flow pores. This article teaches how such beads can be used on a laboratory scale, and how an improved mass transfer is obtained for, in this respect, smaller biomolecules such as proteins. However, as the skilled in this field will realise, results obtained from laboratory scale experiments are not directly applicable on large-scale operations. Thus, it can be assumed that wall ef-

fects in a small column will improve the rigidity of the superporous agarose described, and therefore no reliable conclusions can be drawn as concerns whether or not this porous agarose would be able to withstand the conditions that appear in the large scale columns required for industrial applicability.

Finally, US 5 057 426 (DIAGEN) teaches a method of separation of long-chain nucleic acids from other substances in solutions containing nucleic acids and other materials wherein a matrix denoted a "highly porous" silica gel is used. However, this method has not been shown to provide a sufficient capacity, and therefore there is still a need of improvements within this field.

Summary of the invention

The object of the present invention is to provide a process for isolation of nanoparticles, which avoids one or more of the above-discussed drawbacks. Thus, one object of the present invention is to provide a process for isolation of nanoparticles on a porous matrix, which is more efficient due to the large dynamically available binding area thereof. Another object of the invention is to provide such a method, wherein the high shear damage normally caused by high flow rates through conventional chromatography matrices is avoided.

Thus, one object of the present invention is to provide a method for plasmid-purification, which is particularly useful in large-scale operation. The objects of the invention are more specifically obtained by the process as defined in the appended claims.

Brief description of the drawings

Figure 1A illustrates, in subsequent optical slices, how plasmid DNA under batch conditions has been bound to the surface of superporous agarose beads with a pore size of 4 μm under batch conditions. Figure 1B is an enlargement of an optical section in the middle of the bead, which shows the binding of plasmid DNA to the outer surface of the bead.

Figure 2A and 2B show the same type of prototype bead as under Figure 1, with binding of plasmid DNA on the surface and some plasmid DNA in the superpores under dynamic conditions in accordance with the present invention.

Figure 3A shows a prototype agarose particle with a particle size 180-106 μm, wherein the size of the superpores is 15 μm. Fig 3B shows the results of use of such a prototype as an ion exchanger (hydrophilic Q exchanger), with incubation with plasmid DNA under dynamic conditions

Definitions

The term "nanoparticle " is understood herein to include large molecules and molecule aggregates, such as virus, plasmids, cell organelles, membrane fragments and inclusion bodies. Such nanoparticles are typically of a size within the range of about 30-1000 nm.

The term "dynamic conditions" as compared to static conditions means herein that the process is a chromatographic procedure, wherein the solution comprising the molecules to be isolated is brought to pass over a column packed with a suitable adsorbent or brought in contact with the adsorbent in "expanded bed" mode, as compared to incubation with the adsorbent in batch conditions.

The term "eluant" is used herein with its conventional meaning in chromatography, i.e. a solution capable of perturbing the interaction between the solid phase (adsorbent matrix) and product (nanoparticle) and promoting selective dissociation of the product from the solid phase.

It is to be understood that any term used in the present specification, but not specifically defined herein, is to be construed in accordance with the general meaning understood by those skilled in the present technical field.

Detailed description of the invention

A first aspect of the present invention is a process for isolation of nanoparticles from a solution, comprising the steps of

(a) providing a solution comprising the nanoparticles;

(b) adsorbing the nanoparticles to a superporous matrix by passing the solution through a chromatographic column; and

(c) optionally washing the column with a suitable solution;

wherein the superporous matrix is comprised of particles of an average superpore diameter of up to about 25 μm and the adsorption step is run under dynamic conditions.

Accordingly, the present invention describes for the first time the unexpected advantages of using a superporous matrix under conditions of dynamic flow.

In one embodiment, the process according to the invention is an isolation of nanoparticles expressed in cells, and, consequently, it also comprises a first step of disintegrating the cells to provide the solution comprising nanoparticles. Such disintegration is performed e.g. by lysis, such as alkaline lysis, according to standard protocols (see e.g. Maniatis, T, Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY).

In another embodiment, the present process comprises the further step of eluting the nanoparticles by contacting a suitable eluant with said matrix. Thus, the elution step can be performed as a dynamic or batch procedure. As the skilled person will realise, the nature of the eluant will depend upon the matrix material used in the column, as discussed in more detail below. (For a general review of chromatographic separation, see e.g. Protein Purification Handbook, 1999, Amersham Pharmacia Biotech AB, Uppsala, Sweden)

Thus, the process according to the invention is utilised e.g. for purification of nucleic acids for use in gene therapy and laboratory studies related to gene therapy. In an advantageous embodiment, the present process will provide isolated nanoparticles in the

form of viruses or plasmids of acceptable gene therapy grade. More specifically, it is predicted that in a near future, there will be an increasing demand of virus and plasmids in large quantities for use in gene therapy as carriers or vectors of genetic material. As mentioned above, the previously described methods for isolation of such carriers have not been satisfactory to this end, and the process according to the present invention is thus the first to enable large scale processing of nanoparticles for medical and diagnostic use. (For a general review of important aspects in relation to the purification and use of viral vectors in gene therapy, see e.g. Braas et al., Bioseparation 6:211-228, 1996: Strategies for the isolation and purification of retroviral vectors for gene therapy.)

A further application of the invention is in the preparation of vaccine, in which case molecular aggregates, which are of interest as carriers of immunogenic structures are isolated by the present process. In this case, it is especially important to be able to efficiently isolate well-characterised aggregates having defined properties.

Yet another field of application of the invention is in the context of inclusion bodies, i.e. condensed protein aggregates, which is often the form proteins are obtained in during an efficient expression thereof in a cell system.

As mentioned above, the average superpore diameter of the superporous matrix particles used in the present process may be of a value of up to about 25 μm, for example a diameter within a range of up to 10, such as about 8, or within a range of 10-20, such as about 15 μm. In an alternative embodiment, the superpore diameter is up to about 20-30 or even about 30-40 μm. In the present context, it is to be understood that the term "superporous" relates to particles wherein the pores are large enough so as to constitute an essential part of the structure of the particles, i.e. to penetrate the particles to a much deeper extent than in normal particles for chromatography, which are more or less equally porous straight through the particle. The pores of a normal particle are much smaller than the size of a nanoparticle. The consequence of this is that a nano-

particle only has access to the outer surface of a particle, or to a very thin outer layer due to statistical fluctuation of the pore diameter.

The lower limit of average superpore diameter of the superporous matrix particles used in the present process may be about 4 μm, but under certain conditions, it may be even lower, such as about 2 μm or even about 1 μm. Conditions that may need to be considered when working at these extremes of the diameter range is e.g. the size of the nanoparticle used, the flow rate etc. As regards the nanoparticle size, as the skilled person in this field will realise, a too small pore will not allow a sufficient penetration. On the other hand, a too large pore may also be undesired, since then a reduction in available pore surface area may be expected. Also, the method of preparing the matrix can have an influence on the separation properties, since some methods may give rise to broader pore size distributions or more inhomogeneous pore size distribution over the particle radius pores of a slightly more "pointed" nature than others. However, this last-mentioned factor is not easily predicted and therefore the question whether or not a superporous matrix in this lower range region is working for a defined purpose is most advantageously tested by routine experiments, which are easily performed by a skilled in this field. Binding properties i.e. to which extent the desired nanoparticle has entered the superpores and been bound to the matrix surface can for example be performed as described in the section "Experimental part" below. In specific embodiments, the present invention relates to a separation matrix in which the superpores are penetrated and bind nanoparticles to at least about 25%, preferably at least about 50% and most preferably at least about 75%, such as about 90% or even 95% of the maximum binding capacity.

As regards the flow rate, the skilled person can similarly to what is discussed above easily decide on a suitable flow rate for each selected combination of superporous matrix/desired nanoparticle. Naturally, the flow rate cannot be too high, since then the particles may collapse and/or the nanoparticles may be broken due to high shear forces, and it must also be sufficiently low to allow the desired mass transfer to take place. However, as unexpectedly shown by the present inventors, if batch conditions

are used, i.e. if no flow is applied, then the nanoparticles will not be able to penetrate the superpores to a sufficient degree. This surprising finding is clearly illustrated in the appended drawings, and is discussed in more detail in connection therewith.

US 5 057 426 (DIAGEN) relates to a method of separation of long-chain nucleic acids from other substances in solutions containing nucleic acids and other materials wherein a matrix denoted a "highly porous" silica gel is used. It appears that "highly porous" is defined as pores of a diameter of about 0.05-2.5 μm in particles having a size of about 15-250 μm. Accordingly, the proportion between pore diameter and particle size disclosed therein is in general much smaller than that of the present invention. Thus, the pore diameter of the US 5 057 426 matrix will enable binding of nanoparticles only to the surface layers thereof, and not in the pores, which renders their properties very different from those of the present superporous particles. In particular, the available binding area of said matrix will be considerably smaller than that according to the present invention, wherein the porosity allows binding throughout essentially all of the particles, as illustrated in the drawings.

In one embodiment of the process according to the invention, the nanoparticles are adsorbed to a superporous matrix comprised of particles of a mean size in the range of about 50-300 μm, e.g. within a range of 50-100, 100-200 or 200-300 μm, and especially in a range of about 106-180 μm. However, the particles can advantageously be prepared in any size for which commercially available sieve equipment is exist, such as 250, 212, 180, 150, 125, 106, 90, 75, 63, or 45 μm.

In a preferred embodiment, the super pore diameters of the particles used according to the invention are in the area of about 1/9 of the particle diameter.

The particles used in accordance with the present invention can easily be designed by conventional methods based on values of superpore and particle size as mentioned above. Materials and binding function will be discussed in more detail below. For a definition of the method of preparation used in the examples below, see P.-E. Gus-

tavsson, P.-O. Larsson, J. Chromatogr. A 734 (1996) 231-240, wherein verification was by size exclusion experiments with 0.5 μm latex particles and microscopy.

As mentioned above the present invention shows to be advantageous for the isolation of plasmids. The invention combines a large surface area with the possibility of adsorbing e.g. plasmids in the matrix' pores with a very low shear stress, using chromatography separation performed under dynamic conditions on a matrix of superporous particles. This opens the possibility of retaining a plasmid in its supercoiled form, which is the preferred conformation for gene therapy purposes.

Plasmids isolated in accordance with the invention can be of any origin. Most commonly, microorganisms like bacteria, such as *E.coli*, are used for culturing the plasmids, but the use of host cells is not limited and can be prokaryotic or eukaryotic cells. The host cells harbouring the plasmid can be cultivated in a number of ways well known in the art, e.g. in incubator, bioreactor, fermentor etc. The plasmid isolated according to the invention can be of virtually any size, e.g. in the range of about 2kb up to about 10 kb. At the time of the filing of the present application, commercial plasmids are often of a size of about 300-1000 nm. As an upper limit, the isolation of cosmids and artificial chromosomes is also encompassed, the size of which may be up to about 50 kb and 500 kb, respectively.

Plasmids can be of a high copy number or low copy number and can carry any gene, either genomic or synthetic, encoding protein or peptide of interest, from any source. The culturing of the host cells, as well as the exploitation of the plasmid for gene therapy, is well known in the state of the art.

After culturing the host cells containing the plasmid, the cells are recovered by e.g. centrifugation or filtration. The cell can be stored, for example in a freezer, or processed immediately.

As mentioned above, when the nanoparticle according to the invention has been produced in a cell, lysis thereof is advantageously performed by alkaline lysis. The lysate may then be treated with metal ions, such as of divalent alkaline earth metal ions, to precipitate impurities and specifically RNA and chromosomal DNA. When the precipitated material has been removed, the solution can be applied to the column. (For a detailed disclosure of metal ion precipitation methods in this context, see e.g. WO9916869 in the name of Amersham Pharmacia Biotech.)

As mentioned above, in one embodiment, the nanoparticle isolated according to the present invention is a virus, such as an adenovirus, an adeno-associated virus (AAV), a Herpes Simplex virus (HSV), a retrovirus, etc. In this embodiment, the size of the nanoparticles is in a range of about 20-500 nm. The virus may initially be present in any solution, which depending on the production technology used may comprise a large number of proteins and possibly cell debris. One method of producing viruses is by injecting a small number of viruses in a fertilised chicken egg and allowing the virus to multiply for a number of days, after which the contents of the egg is collected. Thus, in this case the virus produced will need to be purified from the content of the egg. Alternatively, virus can also be produced in cell culture. If the virus is non-lytic, it has to be purified from the culture medium contents (e.g. fetal calf serum, proteins, growth hormones, vitamins, salts, cellular excretion products). If the virus on the other hand is lytic, cells are lysed by the virus, which will mean that all cell content (proteins, genomic DNA, cell organelles) and cellular debris are present in the solution. The further processing thereof can then be performed as described above in relation to plasmids grown in cells.

The adsorbing or binding groups present on the matrix material can be ion exchanging groups, affinity groups, hydrophobic interaction groups, etc. Since the nanoparticles contemplated by the present invention are predominantly negatively charged, positively charged binding groups constitute suitable ligands for the present isolation. However, other types of ligands can alternatively be used.

A matrix useful in the present method can be made of a native polymer such as agarose or a synthetic polymer such as polystyrene/divinylbenzene. In a specific embodiment, the matrix used according to the invention is made from an inorganic material, such as silica. Many such matrix materials are known to the skilled person in this field and various methods are available for producing porous matrices of a desired porosity thereof.

In an advantageous embodiment, the matrix material used is provided with anion exchanging groups. The anion group attached to the matrix can vary from quaternary amino groups (Q), quaternary aminomethyl- (QMA), quaternary aminoethyl- (QAE), triethyl aminomethyl-(TEAE), triethyl aminopropyl- (TEAP), polyethyleneimine- (PEI), diethyl aminoethyl- (DEAE), polyaminoethyl groups (PAE) and others.

In one embodiment of the present invention, the anion exchange groups are bound to the base matrix via extenders such as described in SE 9700383-4. The positive effect caused by such an extender is believed to depend on the fact that the extender will provide the inner surfaces (pore surfaces) and/or outer surfaces of the matrix beads with a flexible polymer layer which is permeable to macromolecules and other molecules are allowed to pass the bed. This will cause an increase in the effective interaction volume as well as in the steric availability of the anion exchange groups. This in turn will increase the mass transfer rate as well as the total capacity.
Suitable extenders should be hydrophilic and contain a plurality of groups selected from e.g. hydroxy, carboxy, amino, repetitive ethylene oxide ($-CH_2CH_2O-$), amido etc. The extender may be in the form of a polymer. Hydrophilic polymeric extenders may be of synthetic origin or of biological origin. Typical synthetic polymers are polyvinyl alcohols, polyacryl- and polymethacrylamides, polyvinyl ethers etc. Typical biopolymers are polysaccharides, such as starch, cellulose, dextran, agarose etc. The preferred polymeric extenders are often water-soluble in their free state, i.e. when they are not attached to the base matrix.

The length (size) of the optimal extender will depend on several factors, such as number of attachment points to the base matrix, type of extender, type and size of anion groups etc. For polymeric extenders for which attachment and/or cross-linking is possible at several monomeric units, it is believed that larger extenders are preferred. It is believed that the most suitable polymers should contain at least 30 monomeric units, which for polysaccharides like dextran indicates a $M_w > 5000$ g/mole.

The base matrix of the beads may be of organic or inorganic nature. Usually it is a polymer, such as glass, a synthetic polymer or a biopolymer. The base matrix may be a hydrophilic polymer such as styrene-divinyl benzene copolymer, which has been hydrophilised on inner and/or outer surface by being coated with the appropriate hydrophilic polymer or by other means. Alternatively, the base matrix may be a water-insoluble hydrophilic polymer, e.g. agarose, cellulose, dextran, starch, etc. which has been cross-linked to give the desired porosity and stability, if necessary. A preferred base matrix in the present invention is based on cross-linked agarose with dextran as extenders.

The eluant used in the present process depends on the nature of the adsorber used in the matrix as well as on the nanoparticles bound thereon. The principle for choosing a suitable eluant is easily made by the skilled person in this field, see e.g. Protein Purification Handbook, 1999, Amersham Pharmacia Biotech AB, Uppsala, Sweden).

One advantage with the present invention is the relatively high flow rates that are contemplated by the superporous matrix, which exceed the flow rates obtainable simply by its gravitational force. It is especially advantageous to be able to use such flow rates without impairing nanoparticles of specific conformations, such as supercoiled plasmids. This feature renders the process especially advantageous in the context of plasmid isolation on an industrial scale.

The present process may be performed with the matrix as an expanded bed or as a packed bed. In packed bed adsorption, the adsorbent is packed in a chromatographic

column and all solutions used during a purification process flow through the column in the same direction. In expanded bed adsorption however, the adsorbent is expanded and equilibrated by applying a liquid flow through the column. A stable fluidised expanded bed is formed when there is a balance between particle sedimentation or rising velocity and the flow velocity during application of the sample and washing steps. In the elution step, the adsorbent is sedimented and behaves like a packed bed adsorbent.

In a second aspect, the present invention relates to a kit for isolation of nanoparticles, which comprises a superporous matrix in a chromatographic column. The matrix is comprised of particles of a superpore diameter of at least about 4 μm, such as about 5-10 or 10-20 μm, and may be of a value of up to about 25 μm, such as about 20-30 or even about 30-40 μm.

In one embodiment, the matrix particles are of a mean size in the range of about 50-300 μm, e.g. within a range of 50-100, 100-200 or 200-300 μm, and especially in a range of about 106-180 μm. However, the particles can advantageously be prepared in any size for which commercially available sieve equipment is exist, such as 250, 212, 180, 150, 125, 106, 90, 75, 63, or 45 μm. Further details regarding the nature of adsorbent coupled to the superporous matrix kit, uses etc are as discussed above in relation to the process.

Detailed description of the drawings

Figure 1A displays following two-dimensional confocal microscopy images (Leica TCS SP confocal scanning laser microscope, argon-krypton laser) obtained after the plasmid DNA with fluorescent dye (TOTO-3, dimeric cyanine nucleic acid stain from Molecular Probes; T-3604)) is incubated with a superporous agarose particle of 50 μm with a pore size of 4 μm under batch conditions for 90 minutes (see example). As can be seen on the images, the 6.1 kbp long circular plasmid DNA is not capable of entering the superpores of the agarose bead under these conditions, but adsorbs to the outer layer of the particle. The enlargement in Figure 1B more clearly shows the lack of plasmid DNA in the pores of the bead.

Figure 2A however, shows the presence of the same stained plasmid DNA as in the above Figure bound in the superpores under dynamic conditions according to the invention in packed bed with superporous agarose particles with a size ranging from 45 to 75 μm and superpores of 4 μm, as described in example 1. This indicates that although the plasmid DNA is not able to enter the superpores under the conditions cited for Figure 1, under the dynamic conditions as described in the experimental part, the plasmid DNA is capable of penetrating the 4 μm superpore. This is more clearly demonstrated in Figure 2B, an enlargement of the confocal microscopy image shown in Figure 2A.

Figure 3A shows a confocal image of prototype agarose particle with a particle size 180-106 μm, wherein the size of the superpores is 15 μm. Fig 3B shows the results of use of such a prototype as an ion exchanger, more specifically a hydrophilic Q exchanger, with incubation with plasmid DNA under dynamic conditions.

Figure 3B shows a fluorescence intensity profile obtained with plasmid DNA visualised with TOTO-3. With dynamic conditions, plasmid DNA utilised the whole particle volume for adsorption, and completely entered the superpores.

Below, the present invention will be described by way of examples provided only as an illustration and not to be construed as limiting the scope of the invention as defined by the appended claims in any way. All references included below or elsewhere in the present application are hereby included herein by reference.

EXPERIMENTAL

Preparation of superporous agarose beads

Superporous agarose beads were prepared by a double emulsification procedure. One hundred ml of an agarose solution (Sepharose quality) (6%, w/v) was prepared by heating a suspension of agarose in water to 95-100°C in a microwave oven, and keep-

ing it at that temperature for 1 min. During the warm-up period, care was taken to keep the agarose powder well suspended with occasional shaking. The solution was transferred to a thermostatic (60°C) stirred glass reactor and a mixture containing 3.0 ml of Tween 80 (Merck-Schuchardt, Munich, Germany) and 50 ml of cyclohexane (Merck, Darmstadt, Germany) (60°C) was added. The mixture was emulsified by stirring at 1000 rpm for 4 min (emulsion 1). A thermostatic solution (60°C) containing 300 ml of cyclohexane and 12 ml of Span 85 (sorbitane trioleate; Fluka, Buchs, Switzerland) was added. The reactor was stirred at 600 rpm. After 1 min the reactor thermostat setting was changed to 25°C. When the temperature decreased below approximately 40°C, the agarose solidified into superporous spherical particles. The particles were isolated on a sieve and washed with water, ethanol-water (50:50, v/v) and finally water. The particles were sized wet with graded metal screens [Gustavsson et. al. J. Chromatogr. A 734/2 (1996) 231-240].

As the skilled person in this field will realise, the superporosity will be dependent on the ratio of organic phase and agarose phase in the preparation procedure of superporous particles. The superpore diameter can be varied independently on the superpore volume and vice versa.

Preparation of RNA and plasmid DNA

RNA and plasmid DNA are prepared using the conventional alkaline lysis protocol followed by anion exchange chromatography and an isopropanol precipitation. Pelleted bacteria are first resuspended, and subsequently lysed under alkaline conditions. After neutralisation, proteins, cell debris and genomic DNA is removed by centrifugation. The supernatant is then loaded on an ion-exchanger column and eluted with a continuous salt gradient, after which the plasmid DNA is precipitated by the addition of isopropanol followed by centrifugation.

Alkaline lysis

The starting material is a 500 ml overnight bacterial cell culture. The suspension is centrifuged at 10.000 rpm in a Sorvall GSA-rotor for 15 minutes at 4°C (10.000g). The supernatant is removed by carefully decanting, and the centrifuge tubes are stored

in inverted position on a piece of paper tissue for 5 minutes to remove all remains of the supernatant. The bacterial pellet is then resuspended by pipetting repeatedly up and down in 50 ml of a buffer pH 8,0 containing 50 mM Tris-HCl, 10 mM EDTA, and 100 µg/ml RNase A. When the bacteria are completely resuspended, 50 ml of a lysis buffer consisting of 200 mM NaOH and 1% SDS is added, and the solutions are thoroughly mixed by gentle inversion of the tube. Lysis is performed by incubation for 5 minutes at room temperature. After this incubation period, 50 ml of a cold neutralisation buffer (3 M KAc pH 5,5) is added to the viscous solution and again the solutions are mixed gently but thoroughly by inverting the tube about five times. White fluffy material containing genomic DNA, proteins and cell debris with SDS is formed, and the lysate becomes less viscous. The fluffy material is removed by 30 minutes centrifugation at 4°C at 11.000 rpm is a Sorvall GSA rotor (20.000g). The supernatant is promptly transferred to a fresh tube and recentrifuged under the same conditions for 15 minutes, after which the supernatant is collected.

Pre-treatment of sample

A column packed with silica gel with DEAE anion exchanger groups is washed with a sufficient volume of equilibration buffer (750 mM NaCl, 50 mM MOPS pH 7,0, 15% isopropanol). The supernatant from the last centrifugation step is then applied to the column. After washing the column with large amounts of 50 mM MOPS pH 7,0 containing 1 M NaCl and 15% isopropanol, the plasmid DNA is eluted from the column by a buffer containing 1.25 M NaCl, 50 mM TRIS pH 8,5 and 15% isopropanol.

The DNA is precipitated by the addition of 0.7 volumes of isopropanol at room temperature. The solution is mixed and subsequently centrifuged at 4°C at 11.000 rpm is a Sorvall SS-34 rotor (15.000g). The supernatant is carefully removed, and the pellet is washed by adding 7 ml of room temperature 70% ethanol. Again the mixture is centrifuged at 4°C at 11.000 rpm is a Sorvall SS-34 rotor (15.000g). Cautiously the supernatant is removed and the tube is left to air-dry for 20 minutes. Finally, the DNA pellet is resuspended in 50 µl of TE-buffer (10 mM Tris-HCl pH 8,0 with 1kh mM EDTA).

The obtained solution containing plasmid DNA is used for incubation with the superporous beads.

Chromatography with superporous adsorbent

Under dynamic conditions, HR5/2 columns (5 mm diameter, 20 mm length; Amersham Pharmacia Biotech) are packed with several types of derivatized agarose beads with a particle size of ranging between 45 and 180 µm and superpores of 4 or 15 µm diameter. The columns are installed on an Äkta Explorer 10 (Amersham Pharmacia Biotech). In a first stage, the columns are washed with 1 M NaOH, at a speed of 90 cm/h, followed by an equilibration with 5 column volumes of equilibration buffer (20 mM Tris-HCl, pH 8,0, 10 mM EDTA). Blank samples are taken by opening the columns and transferring 20 µl of agarose beads to different tubes. In the next step, a 50 µl sample of the plasmid DNA preparation (1:4 diluted with H_2O) is loaded on the columns at the same flow rate as described above. Again, the columns are opened and a 20 µl sample is taken for confocal scanning laser microscopy.

Visualisation of plasmid DNA adsorbed to individual chromatography adsorbent particles by confocal scanning laser microscopy.

Instrumentation

Confocal microscopy measurements were performed with a Leica TCS SP confocal scanning laser microscope equipped with an argon-krypton laser and with TCS SP NT software for image evaluation.

Adsorbents, plasmid DNA and chemicals

* Superporous agarose particles (Prototype agarose particles with particle size 45-75 µm, size of the superpores 4 µm, hydrophilic Q exchanger)
* Superporous agarose particles (Prototype agarose particles with particle size 106-180 µm, size of the superpores 15 µm [Gustavsson et. al. J. Chromatogr. A 734/2 (1996) 231-240]; hydrophilic Q exchanger)
* Plasmid DNA pUc18 with a 3.5 kbp insert. Total size is 6.1 kbp.

* TOTO-3, dimeric cyanine nucleic acid stain (Molecular Probes T-3604)

50 µl TOTO-3 reagent was diluted in TE-buffer to 20 ml

* TE-buffer: 10 mM TRIS, 1 mM EDTA, pH 7.5

* NAP-5 column (Sephadex G-25 DNA grade, Amersham Pharmacia Biotech)

Staining of plasmid DNA with TOTO-3 and batch incubation.

50 µl plasmid DNA was mixed with 50 µl TE-buffer and 50 µl TOTO-3 solution. Incubation was then performed by end-over-end mixing for 60 minutes, whereupon the reaction mixture was desalted with TE-buffer on a NAP-5 column. Gel particles (the different agarose prototypes respectively) were washed two times with TE-buffer by repeated dilution, centrifugation, and removal of supernatant. 400 µl DNA-TOTO-3 was then incubated end-over-end with 10 µl gel slurry (1:1) for 90 minutes. The particles were then analysed by confocal microscopy.

Staining of plasmid DNA adsorbed to superporous agarose particles.

Plasmid DNA was adsorbed under dynamic conditions in a column experiment to superporous agarose particles (in 20 mM TRIS-HCl, 10 mM EDTA pH 8.0). 16 µl gel slurry (1:1 in TOTO-3 solution) was incubated end-over-end for 2.5 hours. The particles were then analysed by confocal microscopy.

Confocal microscopy

Individual particles were analysed by acquisition of horizontal sections[1] [Ljunglöf et. al. J. Chromatogr. A 844 (1999) 129-135] and section series[2]. The objectives used for confocal scanning were 20x/0.7 and 63x/1.2 (both water immersion lenses). The laser provided excitation of TOTO-3 at 647 nm and the emitted light was detected between 650 and 800 nm. To reduce background fluorescence and noise, the images were generated by accumulating 8 scans per image.

[1] Two-dimensional confocal images perpendicular to the optical axis.

[2] A stack of horizontal sections separated in space to describe a three-dimensional volume.

CLAIMS

1. A process for isolation of nanoparticles from a solution, comprising the steps of

(a) providing a solution comprising the nanoparticles;

(b) adsorbing the nanoparticles to a superporous matrix by passing the solution through a chromatographic column; and

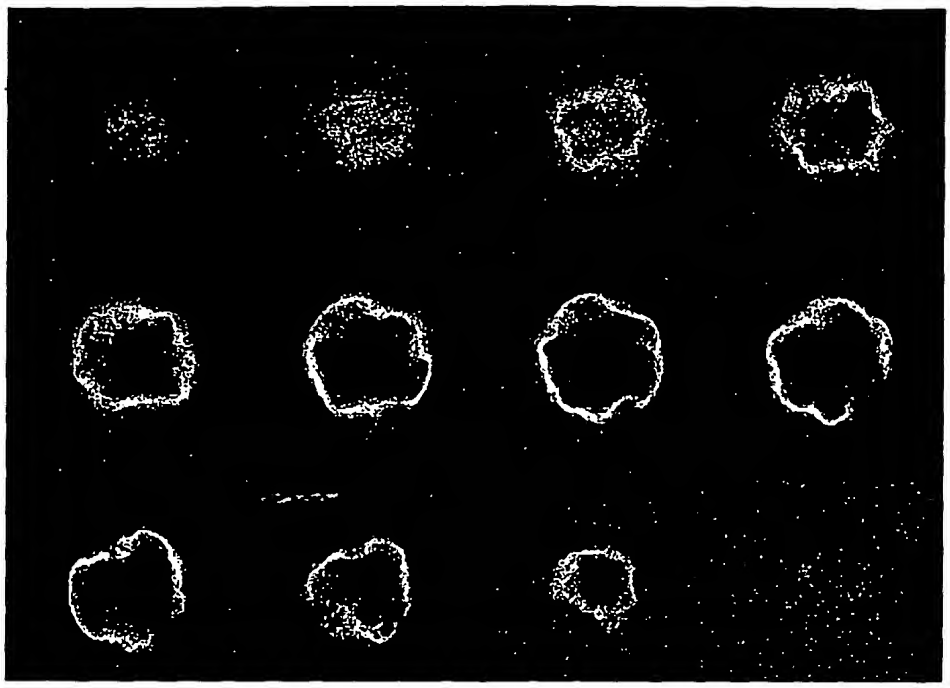
(c) optionally washing the column with a suitable solution;

wherein the superporous matrix is comprised of particles of an average superpore diameter of up to about 25 μm and the adsorption step is run under dynamic conditions.

2. A process according to claim 1, which comprises a first step of disintegrating cells, wherein the nanoparticles are expressed, to provide the solution comprising nanoparticles, which disintegration is a lysis.
3. A process according to claim 1 or 2, which comprises a further final step of eluting the nanoparticles by contacting a suitable eluant with said superporous matrix.
4. A process according to any one of the previous claims, wherein the mean size of the particles of said superporous matrix is in the range of about 50-300 μm.
5. A process according to any one of the previous claims, wherein the nanoparticles are plasmids or viruses.
6. A process according to any one of the previous claims, wherein the matrix is agarose derivatized with binding ion exchanging groups.
7. A process according to any one of the previous claim, wherein the solution is recirculated over the column at least once.

Figure 1

A)



B)

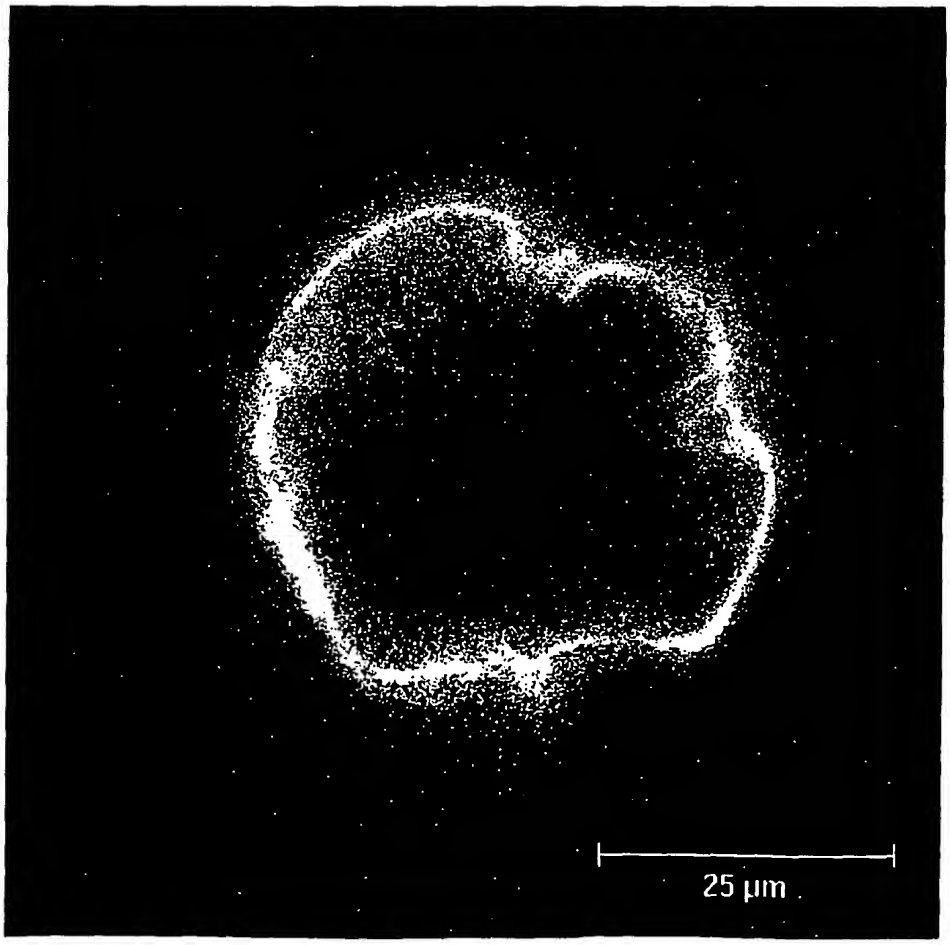
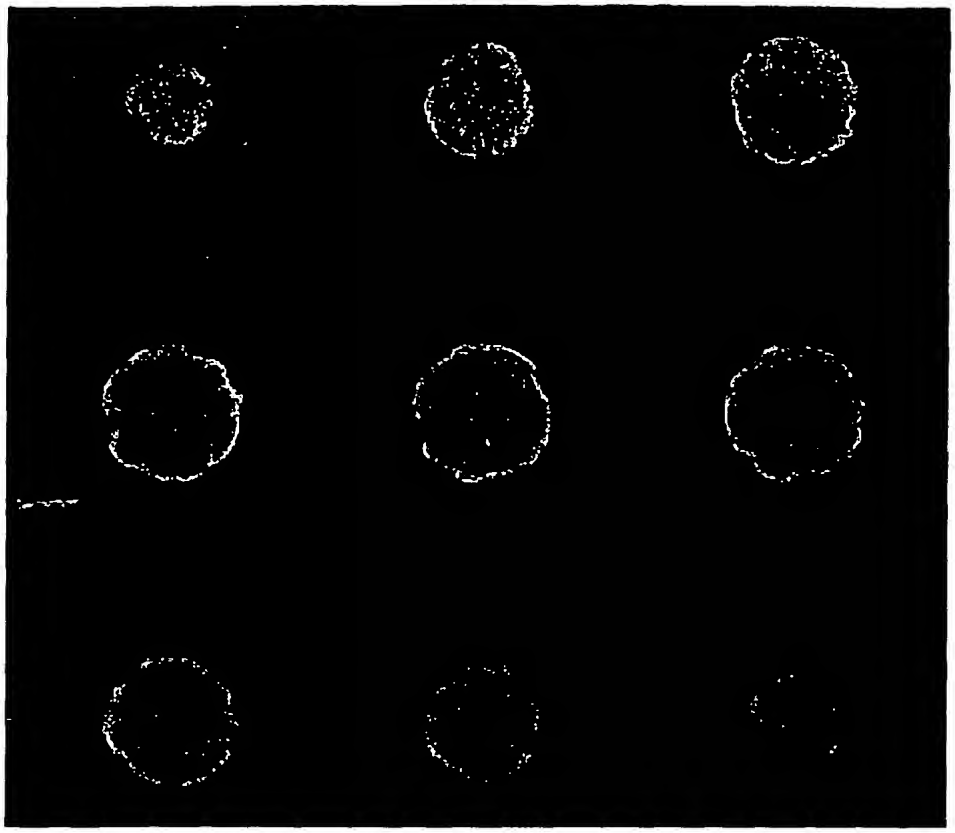


Figure 2

A)



B)

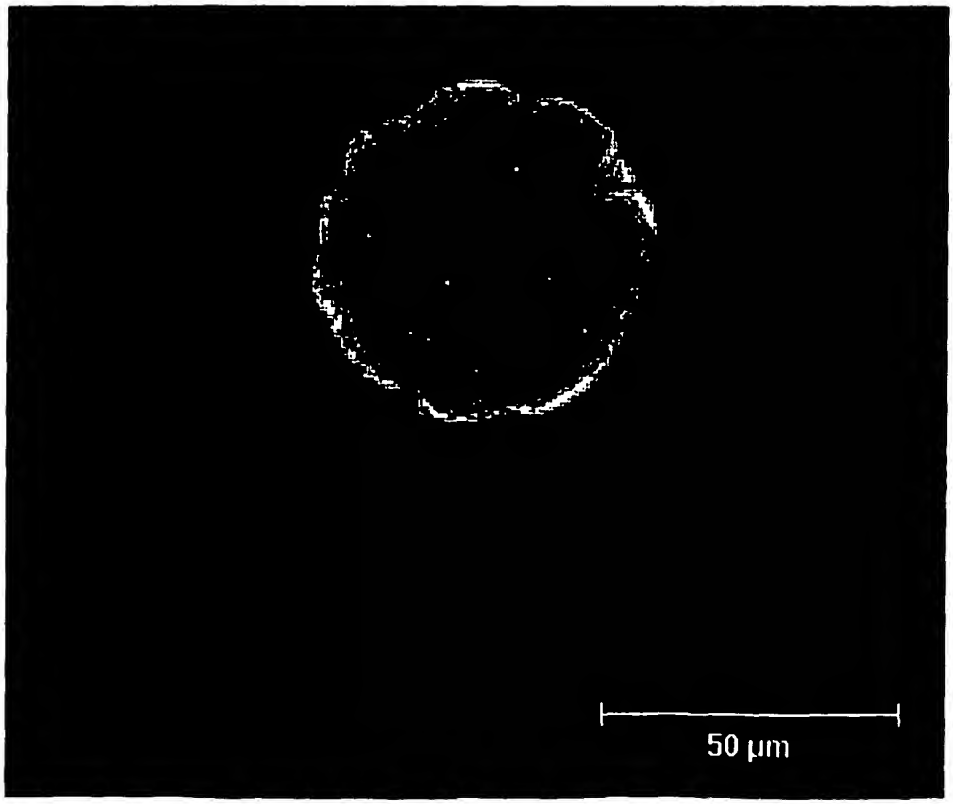
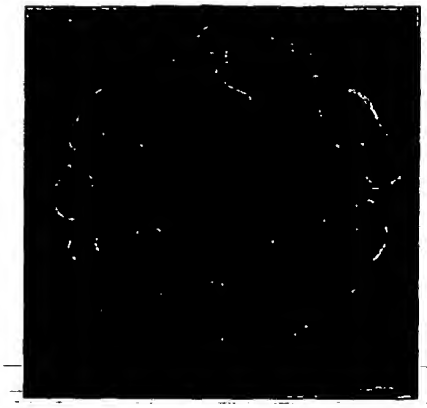
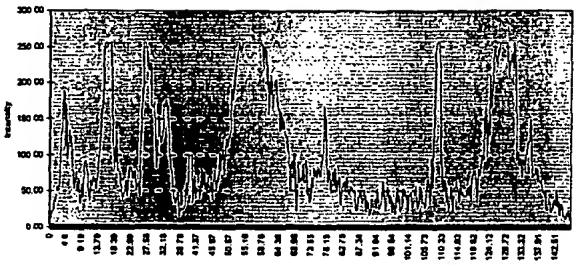


Figure 3

A)



B)



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/00764

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01D15/08 C12N15/10 C12N7/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01D C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 07597 A (INST SUPERIOR TECNICO) 1 February 2001 (2001-02-01) cited in the application the whole document	1-7
X	US 5 723 601 A (P.O. LARSSON) 3 March 1998 (1998-03-03) column 2, line 44 - line 47 column 7, line 58; table 1 column 7, line 65; table 1 column 9; example 7	1,3,6

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

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'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 2002	04/07/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hilgenga, K

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/00764

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAI A ET AL: "Enhanced performance of expanded bed chromatography on rigid superporous adsorbent matrix" JOURNAL OF CHROMATOGRAPHY, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 867, no. 1-2, 21 January 2000 (2000-01-21), pages 113-130, XP004252935 ISSN: 0021-9673 abstract page 128, column 2, line 27 - line 38 ---	1,3,4
X	COLPAN M ET AL: "HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF HIGH-MOLECULAR-WEIGHT NUCLEIC ACIDS ON THE MACROPHOROUS ION EXCHANGER, NUCLEOGEN" JOURNAL OF CHROMATOGRAPHY, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 296, 1984, pages 339-353, XP000960881 ISSN: 0021-9673 page 349, last paragraph ---	1-3,5
X	GUSTAVSSON P-E ET AL: "Superporous agarose beads as a hydrophobic interaction chromatography support" JOURNAL OF CHROMATOGRAPHY A, ELSEVIER SCIENCE, NL, vol. 830, no. 2, 15 January 1999 (1999-01-15), pages 275-284, XP004153874 ISSN: 0021-9673 page 276, column 2, line 4 - line 19 ---	1,3,4
X	GUSTAVSSON P-E ET AL: "Superporous agarose, a new material for chromatography" JOURNAL OF CHROMATOGRAPHY A, ELSEVIER SCIENCE, NL, vol. 734, no. 2, 24 May 1996 (1996-05-24), pages 231-240, XP004039112 ISSN: 0021-9673 cited in the application the whole document ---	1,3,4,6
X	US 5 057 426 A (K. HENCO) 15 October 1991 (1991-10-15) cited in the application column 9, line 10 - line 15; claims 1,2 -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

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